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Identification of molecular signatures and pathways common to blood cells and brain tissue based RNA-Seq datasets of bipolar disorder: Insights from comprehensive bioinformatics approach

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ABSTRACT

Bipolar disorder (BD), also referred to as manic depression, is a mental illness marked by significant mood fluctuations as well as changes in sleep, energy, thinking, and behavior. About 1% of the world's population is affected by BD. In the United States, roughly 3% of people are thought to be impacted at some point in their lives; female and male percentages appear to be similar. The most prevalent age for the onset of symptoms is 20 years old; early-onset is connected with a worse prognosis. The primary molecular markers for identification and therapeutic target screening have now become a worldwide concern. We looked at gene expression data from brain tissue (RNA-seq) in this work. Transcriptomic analysis of GSE53239 and GSE81396 was implemented to figure out the DEGs. Gene ontology and Kyoto Encyclopedia of Genes and Genomes analyses were used to perform functional annotation and pathway enrichment analysis of DEGs, respectively. In addition, a protein-protein interaction network was built to find hub proteins (HSPA1A, GNL1, FOSL1, DDX39B, BAG3, ARHGAP11A, MDC1, PRRC2A, VPS52, and DHX16). Subsequently, TFs-DEGs interaction, miRNAs-DEGs interaction, and correlated diseases were also identified. In the brain tissues of BD patients, we discovered possible new linkages between pathogenic processes. Beryllium sulfate exhibited the most negative correlation and ability to reverse BD. As a result, this research provides molecular biomarkers at the RNA and protein levels that may aid in the better understanding of molecular processes, as well as potential pharmacological targets and therapies for the treatment of BD patients.

1. Introduction

Bipolar disorders (BD) are a group of emotional, energetic, and cognitive illnesses defined by biphasic mood swings between mania and psychosis and depressive episodes and manifested as periodic episodes of changes in energy levels and behavior [1]. Bipolar disorder is a serious, long-term chronic illness that is one of the world's top ten causes of disability and afflicts 1% of the world's population [2,3]. According to epidemiological research, the prevalence of BD during a person's lifespan ranges from 2% to 4%, putting a tremendous cost on families and society [4]. According to reports, BD affects 4.4% of the US population [5], with prevalence ranging from 0.1% to 6.0% in European countries [6]. At one year following an index episode, BD has a recurrence risk of over 50%, and at four years, it is over 70% [7,8]. BD is associated with a high death rate; on average, people with BD die 10–20 years sooner than the overall population [9]. As public awareness of juvenile bipolar

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disorder grows, more children and adolescents are identified and treated. In children and teenagers, many of the symptoms of bipolar disorder are comparable to those of attention deficit hyperactivity disorder (ADHD) and other disorders. According to two major cohort studies, BD is the psychiatric disorder with the most unprecedented rates of completed suicide. Patients suffering from BD can suffer from severe medical comorbidities such as endocrine diseases, cardiovascular disease, and drug addiction, with up to 15% of patients committing suicide [10,11]. Mental disorder, a record of suicidal behavior, aggression or impulsivity, a family background of suicide, social alienation, marital issues, economic barriers, and physical health problems are all risk factors. After a long-term follow-up of individuals with BD after psychiatric interaction, a Danish study discovered 5% suicides among women and 8% suicides among men [12]. Studies in the adult literature on twins, adoption and family history show a strong genetic component, with elevated risk by four to six times of disease in first-degree relatives of affected people [13].

Acute stabilization and relapse prevention are typically the two steps of treatment for BD. Acute stabilization is defined as the transition from a manic or depressed phase to a euthymic state; relapse prevention is defined as sustaining the euthymic condition while reducing subthreshold symptoms and improving overall function [14]. The treatment of BD is quite complex, as few treatments have been demonstrated to be effective, and there is considerable debate over the use of antidepressant medicines. Despite treatment, roughly 37% of patients return to depression or mania within a year, and 60% within two years [15]. Syndromal recovery from acute periods of mania or bipolar major depression can occur in as many as 90% of patients who get current therapies. However, full symptomatic recovery takes time, and persistent symptoms of varying intensity and functional consequences are normal [16]. Bipolar disorder is linked to significant morbidity, death, and impairment in psychosocial and occupational functioning in adolescents and adults. IPSRT (Interpersonal and social rhythm therapy) is an empirically supported supplemental treatment for individuals with BD that has been proven to prevent relapse, improve occupational and psychosocial performance, and expedite recovery from a bipolar depressive episode [17]. Cade discovered lithium's anti-manic abilities in Australia in 1949. For nearly 60 years, lithium has remained the very first treatment for manic depression [18]. For the treatment of bipolar disorder, lithium is one of the most regularly used mood stabilizers. Although the particular mechanism(s) by which this mood stabilizer operates is unknown, lithium has recently been linked to neurotrophic/neuroprotective effects. According to new studies, this drug may have novel applications beyond mood stabilization [19–22]. At the same time, there is evidence that genetic load has a role in lithium responsiveness in BD [23].

Despite evidence-based guidelines' recommendations, polypharmacy is nevertheless frequent in BD clinical practice, illustrating the gap between research and everyday clinical practice. The development of effective treatments has been delayed by a lack of understanding of the disorder's underlying pathophysiology and neurobiology [14]. To better understand the pathophysiology of BD, numerous ideas have been offered. It is widely acknowledged, and it appears self-evident, that a rising number of studies concentrating on affective disorders incorporate environmental and genetic factors [24–26]. It has been an important topic of concern among doctors in recent years. New technologies show plenty of potential in providing unique and individualized solutions. However, further intervention research that can help people with BD, caregivers, and educators is still required.

In this research, we have uncovered molecular signatures and pathways at the transcriptional and post-transcriptional phases to understand better the pathogenic factors that affect BD and identify potential biomarkers for early diagnosis. The identification of innovative targeted therapeutics and the development of new pharmacological treatments may be aided by understanding the processes underpinning numerous signaling pathways in the genesis of BD [27]. Using differentially expressed genes (DEGs), we analyzed the transcriptome data set associated with BD and applied statistical techniques to predict hub protein set variability and other pathogenic alterations. Furthermore, we have also carried out protein interactions, transcriptional and post-transcriptional processes, and the selection of prospective drug molecules.

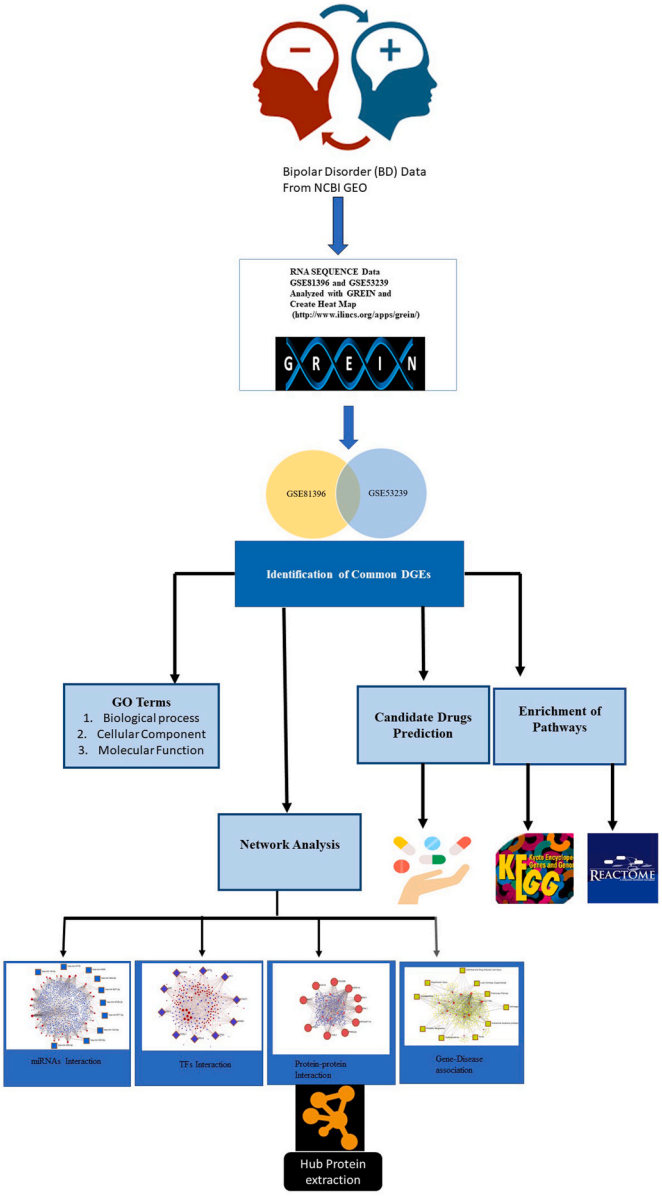


Fig. 1. Schematic illustration of the overall strategies and integrative bioinformatics analytical approach employed in this study.

2. Materials and methods

The whole workflow of the integrated systems biology analytical technique to reveal molecular gene signatures and pathways of BD is depicted in Fig. 1.

2.1. Data mining

Gene expression profiles for RNA-seq datasets GSE53239 [28] and

Table 1
Overview and the quantitative measurements of datasets used in this study.

SL No.	GEO accession	GEO Platform	Sample Size	Control	Case	Number of DEGs		
						Up	Down	Total
1	GSE53239	GPL9115	22	11	11	1228	181	1409
2	GSE81396	GPL11154	16	8	8	996	1091	2087
	Combined Number		38	19	19	2224	1272	3496

GSE81396 [29] gathered from the NCBI (National Center for Biotechnology Information) GEO (Gene Expression Omnibus)[1] database [30–32], a public repository that offers free access to microarray, next-generation sequencing, and other types of high-throughput functional genome data. The GSE53239 dataset was employed on the GPL9115 Illumina Genome Analyzer II (*Homo sapiens*) and the GPL15433 Illumina HiSeq 1000 (*Homo sapiens*), which had 11 cases and 11 controls, for a total of 22 samples. This dataset collected postmortem dorsolateral prefrontal cortex from 11 bipolar patients and 11 healthy people: Otherside, The GSE81396 dataset (GPL11154), including 8 cases and eight control. The samples of this dataset were collected from human putamen and caudate nucleus tissues in normal (control) and BD people (case). However, A total of 38 samples were considered in this study (Table 1). After that, we analyzed these datasets and collected common genes between these two datasets.

2.2. Screening of differentially expressed genes (DEGs)

RNA-sequencing (RNA-seq) is a potent approach for studying the complexities of gene expression of brain tissue [28]. DEGs were analyzed via GREIN[2] [33], which is a web tool that provides choices for exploring RNA-seq data. Important DEGs were filtered based on p-value < 0.05 and $|logFC| > 1.0$. Using an online VENN analysis tool [2] [34], we have obtained the common DEGs between the two datasets (GSE81396 and GSE53239) from both upregulated and down-regulated. These overlapping DEGs were used for the next steps [35].

2.3. Functional enrichment of gene sets

Gene set enrichment analysis is a large-scale research project that categorizes common biological findings such as biological processes or chromosome sites relevant to several interconnected disorders [36,37]. The online bioinformatics tool DAVID[3] [38] (Database for Annotation, Visualization, and Integrated Discovery) v6.8 was used to conduct Gene Ontology (biological processes, cellular components, and molecular functions) [39,40], KEGG pathway (Kyoto encyclopedia of genes and genomes) [41], and Reactome pathway [42] enrichment studies using up-and down-regulated gene sets from the overlapped DEGs, keeping the p-value < 0.05 used as the standard metric [43].

2.4. Protein-protein interaction and hub proteins screening

Protein-protein Interaction (PPI) controls a variety of biological functions, including cell-to-cell connections, metabolic control, and developmental control [44,45]. Using the NetworkAnalyst[4] [46], we get the PPI network based on the physical interactions of DEGs from the STRING database [47]. A confidence score of 600 was determined for the STRING Interactome. To visualize networks and conduct topological research, NetwokAnalyst was employed [48]. Topological analysis was utilized to identify hub proteins using the Cyto-Hubba plugin [49] in Cytoscape software[6] [50]. The CytoHubba used 11 different techniques to determine hub proteins from a PPI network. The top 10 most entangled nodes of the PPI network were found using the degree technique. The top PPI network modules were discovered using the MCODE plugin in Cytoscape.

2.5. Identification of TFs and miRNAs interaction with common DEGs

Transcription factors (TFs) are proteins that regulate gene expression through transcription [51]. In the JASPAR [52] database, an open-access curated, non-redundant collection of DNA binding transcription factors, we used the NetworkAnalyst tool to locate topologically credible TFs that bind to our mutual DEGs [53]. And on the other hand, miRNAs and their target mRNA have perfect or near-perfect complementary binding, which adversely controls gene expression by speeding up mRNA breakdown or reducing mRNA translation [54]. We used the mirTarbase database [55] to look into DEGs-miRNA interactions. Illness connections of miRNAs and their targets have been published in mirTarBase, which may reveal prospective targets for disease diagnostics [56].

2.6. Identification of candidate drugs

One of the most important aspects of this study is the identification of potential drugs. Based on the DEGs, the candidate drug was found utilizing the Drug Signatures database (DSigDB) from Enrichr. DSigDB is a global database for identifying targeted signals [57], and Enrichr is a well-known website with a large number of gene-set libraries that may be used to investigate gene set enrichment on a genome-wide scale [58].

2.7. Analysis of gene-disease association

DisGeNET is a large-scale discovery platform that aims to investigate a wide range of questions related to the genetic basis of human disorders. DisGeNET has around 380,000 connections between over 16,000 genes and 13,000 disorders, making it one of the most extensive databases of its kind available today [59,60]. We used the DisGeNET server through NetworkAnalyst to investigate the gene-disease association to find diseases and chronic problems related to common DEGs [61].

2.7.1. Potential biomarker validation

Biomarkers are critical to the sensible advancement of clinical interventions, but the terminology and concepts involved in their use in research and clinical practice, particularly in the disciplines of chronic illness and nourishment, remain a source of confusion [62]. We verified some biomarkers in our study through literature reviews.

3. Results

3.1. Identification of common differentially expressed genes (DEGs)

Using GREIN, we analyzed two brain tissue RNA-seq datasets (GSE81396 and GSE53239). Using p-value < 0.05 and $|\log FC| > 1.0$ to indicate upregulated and downregulated DEGs, a total of 1409 genes were differentially expressed for GSE53239, having 1228 upregulated and 181 down-regulated. Similarly, we identified the most important DEGs for GSE81396 based on our findings. We detected 996 upregulated genes and 1091 down-regulated genes in the GSE81396 sample, totaling

[1] https://www.ncbi.nlm.nih.gov/geo/ [2] http://www.ilincs.org/apps/grein/.
[2] http://bioinformatics.psb.ugent.be/webtools/Venn/.
[3] https://david.ncifcrf.gov/.
[4] https://www.networkanalyst.ca/ [6] https://cytoscape.org/.

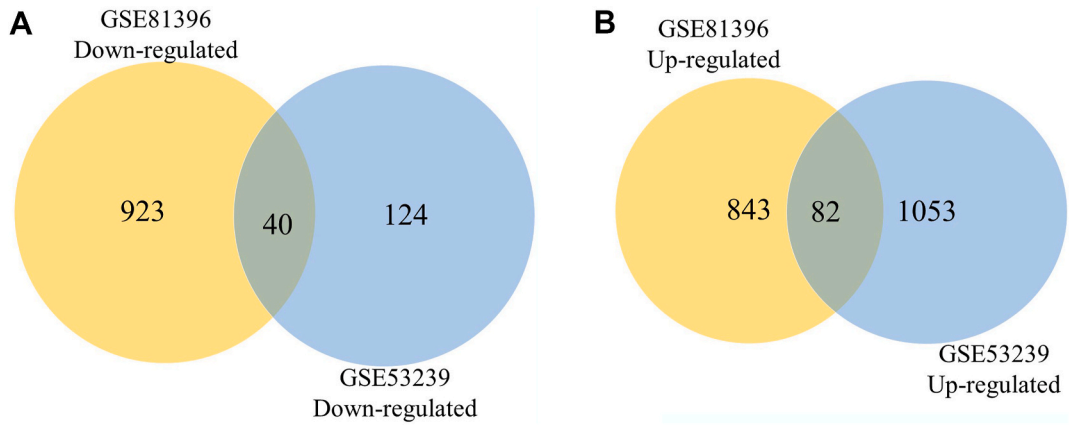


Fig. 2. Differentially expressed genes between two RNA-seq dataset (GSE81396 and GSE53239). A Venn diagram showing a number of unique and common (a) common upregulated genes and (b) common down-regulated genes between two datasets.

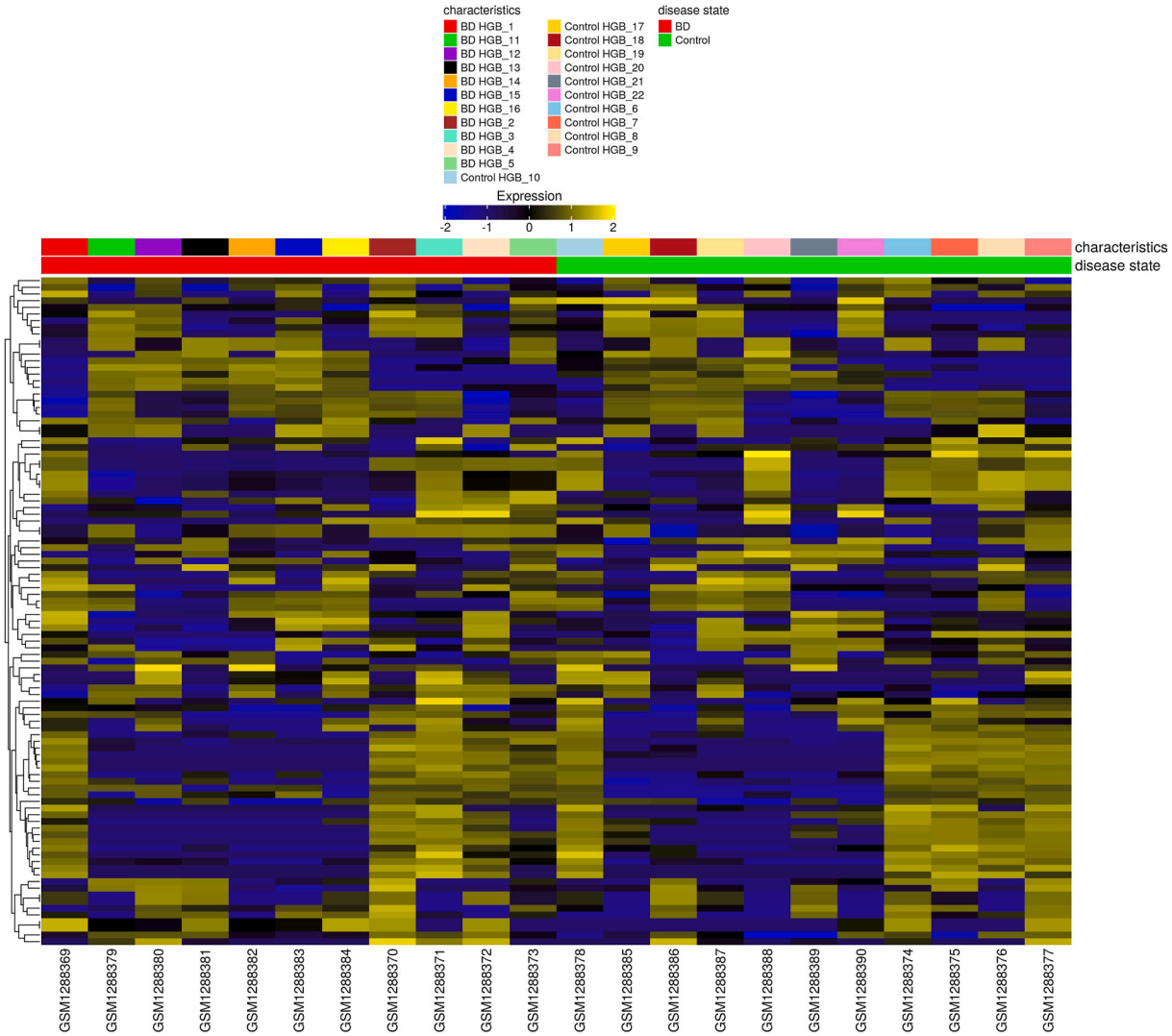


Fig. 3. Hierarchical heatmap clustering of gene expression (GSE53239). In this graph, upregulated gene expressions are denoted by yellow, downregulated gene expressions are denoted by blue and insignificant gene expressions are denoted by black. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2087 genes. We have used a reputable web tool for Venn analysis to find common DEGs. We found 82 upregulated and 40 down-regulated mutual DGEs between the two datasets, a total of 122 common DGEs (Fig. 2). Furthermore, Fig. 3 and Fig. 4 represents thehierarchical heatmap clustering of gene expression of these two datsets.

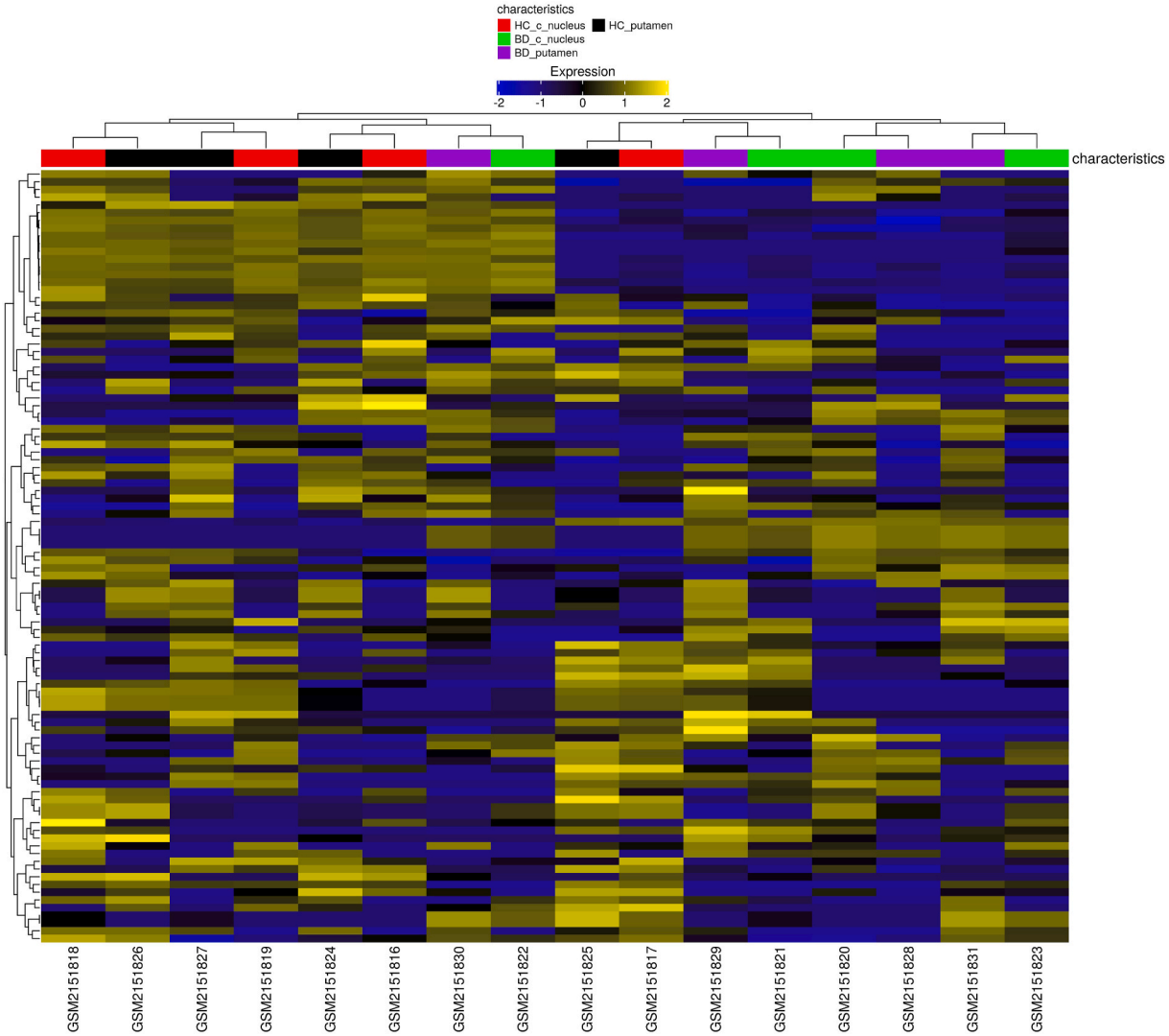


Fig. 4. Hierarchical heatmap clustering of gene expression (GSE81396). In this graph, upregulated gene expressions are denoted by yellow, downregulated gene expressions are denoted by blue, and insignificant gene expressions are denoted by black. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2. Evaluation of functional enrichment

To discover the biological importance and enriched pathways highlighted in this work, we have performed gene ontology and pathway enrichment studies using Enrichr. Gene ontology makes use of gene functions and their components to deliver large amounts of computerized knowledge. An ontology conceptually specifies a body of information within a certain environment [63]. The GO database was used as an annotation source for gene ontology study in three aspects (biological process, molecular function, and cellular component). The top 10 terms in the biological processes, molecular functions, and cellular components categories are summarized in Table 2. In addition, Fig. 5 shows the overall ontological analysis for each category as a bar graph. This graph also demonstrates that interferon-gamma-mediated signaling pathways and cytokine-mediated signaling pathways are among the top Go terms for biological processes. CXCR chemokine receptor binding and MHC class II protein complex binding are the top two GO keywords in the molecular function experiment. Top GO terms for cellular components are MHC protein complex and MHC class II protein complex. For high-throughput molecular data, pathway analysis has become the preferred method for extracting and understanding the underlying biology [64]. The most impacted pathways of common DEGs of BD were gathered from two databases, including KEGG and Reactome. Fig. 6 depicts two pathways in the bar graph.

3.3. Networking of protein-protein interactions and hub proteins screening

After the functional enrichment analysis, a Protein-Protein Interaction network was formed among the overlapped genes. Using NetworkAnalyst and the STRING database, a densely connected scale-free network was built. The resultant PPI network consists of 525 nodes, 561 edges, and 40 seeds from which the top 10 seed proteins were highlighted after visualizing the PPI network in NetworkAnalyst. The PPI network that resulted was also displayed using the Cytoscape software. Protein-protein interaction networks encoded by DEGs were created using degree techniques to uncover the center protein, known as hub genes (HSPA1A, GNL1, FOSL1, DDX39B, BAG3, ARHGAP11A, MDC1, PRRC2A, VPS52, DHX16). Also, these hub genes were recognized as the same top 10 proteins that were highlighted in the PPI network of NetworkAnalyst (Fig. 7 and Table 3). These hub genes can be considered as potential biomarkers that could lead to new therapy options for BD patients. Furthermore, Fig. 8 reprents the PPI network top one module of DEGs that shows the interactions between two genes.

Table 2
The results of a functional enrichment study of degs to find gene ontology keywords in bipolar disorder.

Category	GO ID	Term	P-value
GO Biological Process	GO:0060333	interferon-gamma-mediated signaling pathway	7.56×10^{-12}
	GO:0019221	cytokine-mediated signaling pathway	8.80×10^{-11}
	GO:0071346	cellular response to interferon-gamma	1.39×10^{-10}
	GO:0002478	antigen processing and presentation of exogenous peptide antigen	1.00×10^{-08}
	GO:0002484	antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway	3.97×10^{-08}
	GO:0002486	antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, TAP-independent	3.97×10^{-08}
	GO:0002480	antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-independent	7.91×10^{-08}
	GO:0002483	antigen processing and presentation of endogenous peptide antigen	1.10×10^{-06}
	GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	1.37×10^{-06}
	GO:1990869	cellular response to chemokine	1.43×10^{-06}
Go Molecular Function	GO:0045236	CXCR chemokine receptor binding	2.58×10^{-06}
	GO:0023026	MHC class II protein complex binding	2.58×10^{-06}
	GO:0008009	chemokine activity	7.42×10^{-06}
	GO:0042379	chemokine receptor binding	1.13×10^{-05}
	GO:0032395	MHC class II receptor activity	2.33×10^{-05}
	GO:0048248	CXCR3 chemokine receptor binding	3.41×10^{-04}
	GO:0046978	TAP1 binding	3.41×10^{-04}
	GO:0005125	cytokine activity	3.64×10^{-03}
	GO:0008395	steroid hydroxylase activity	1.91×10^{-02}
	GO:0030292	protein tyrosine kinase inhibitor activity	2.92×10^{-02}
GO Cellular Component	GO:0042611	MHC protein complex	3.00×10^{-20}
	GO:0042613	MHC class II protein complex	3.46×10^{-13}
	GO:0098553	lumenal side of endoplasmic reticulum membrane	3.25×10^{-12}
	GO:0071556	integral component of lumenal side of endoplasmic reticulum membrane	3.25×10^{-12}
	GO:0012507	ER to Golgi transport vesicle membrane	9.58×10^{-10}
	GO:0030662	coated vesicle membrane	1.12×10^{-09}
	GO:0030658	transport vesicle membrane	2.29×10^{-09}
	GO:0030134	COPII-coated ER to Golgi transport vesicle	2.13×10^{-08}
	GO:0030176	integral component of endoplasmic reticulum membrane	1.65×10^{-07}
	GO:0030666	endocytic vesicle membrane	4.09×10^{-07}

3.4. Examining the interaction between TFs and miRNAs

To begin, regulatory elements were discovered, and the transcriptional and post-transcriptional regulatory networks of DEGs were studied using TFs-DEGs and miRNAs-DEGs interactions. There were ten transcription factors (FOXC1, GATA2, YY1, NFIC, SRF, NFKB1, FOXL1, RELA, USF2, TP53) and ten miRNAs (hsa-mir-335–5p, hsa-mir-124–3p, hsa-mir-4459, hsa-mir-627–3p, hsa-mir-34a-5p, hsa-mir-26b-5p, hsa-mir-4768–3p, hsa-mir-4779, hsa-mir-877–3p, hsa-mir-16–5p) found in the regulatory network. Fig. 9 and Fig. 10 represents interaction between TFs and miRNAs.

3.5. Analysis of candidate drugs

The Drug Signatures Database (DSigDB) via Enrichr was utilized to locate small molecule medications based on common DEGs, and the top 10 associated small molecules with very significant correlations were identified. Beryllium sulfate exhibited the most negative correlation and ability to reverse BD among these compounds (Fig. 11 and Fig. 12).

3.6. Gene-disease analysis

Different diseases can be linked or correlated under certain situations, such as sharing one or more genes [65]. Approaches to disorder therapy design reveal the connection between genes and disorders [66]. Schizophrenia, Autosomal recessive propensity, Hypersensitivity, Liver Cirrhosis, Experimental, and Arthralgia are the most coordinated to our identified hub proteins, according to Gene-disease association analysis via NetworkAnalyst (Fig. 13).

3.7. Potential biomarker verification

We conducted literature research to back up our proposed research goals. HSPA1A, GNL1, FOSL1, DDX39B, BAG3, ARHGAP11A, MDC1, PRRC2A, VPS52, and DHX16 were among ten hub proteins linked to BD in our study (Table 3). Dysregulation of these genes can result in severe psychiatric disorders. Table 4 repesents only those biomarkers which are associated with our current study with relevent references.

HSPA1A is a crucial component of the protein quality control system, maintaining proper protein folding, refolding of misfolded proteins, and managing protein degradation targeting [74]. SNPs in the HSPA1A gene have been linked to schizophrenia and BD [67]. It has been associated with neurodegenerative diseases, cell senescence and aging, and inflammatory diseases [68]. The GNL1 gene was revealed in the class I region of the human major histocompatibility complex [69]. FOSL1 is one of the immediate-early genes of bipolar disorder [70]. The human major histocompatibility complex class III region contains the DDX39B gene [71]. Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, schizophrenia, and epilepsy are all linked to this hub gene [72]. The specific macroautophagy system mediated by BAG3 has indeed been shown to play an important function in ensuring the quality of cellular proteins by eliminating proteins capable of aggregation [73]. The BAG3 system can also eliminate the accumulation of proteins connected to neuroprotection in time of life neurodegenerative diseases like ALS, HD, and AD, such as mutant SOD1 or polyQ43-huntingtin [73]. Though BAG3 has not previously been linked to BD, it has been linked to relevant expression changes in animal brains in response to antipsychotic treatment [75]. ARHGAP11A stimulates neuronal migration and lamination in non-cell-autonomous radial glia [76]. PRRC2A is a protein that regulates oligodendrocyte specification and myelination. In a mouse model, Nestin-Cre-mediated PRRC2A knockout can cause a significant lack of myelination, shortened life span, and motor and cognitive deficits. These findings point to a new path for developing therapeutic strategies for hypomyelination-related neurological diseases [77]. The DHX16 protein encodes a DEAD-box RNA-dependent ATPase that mediates ATP hydrolysis during pre-mRNA splicing [78].

4. Discussions

BD is one of the worst causes of disability with inadequate therapy and a molecular reason that remains a mystery [3]. This study investigated regulatory patterns, molecular key pathways, and protein-protein

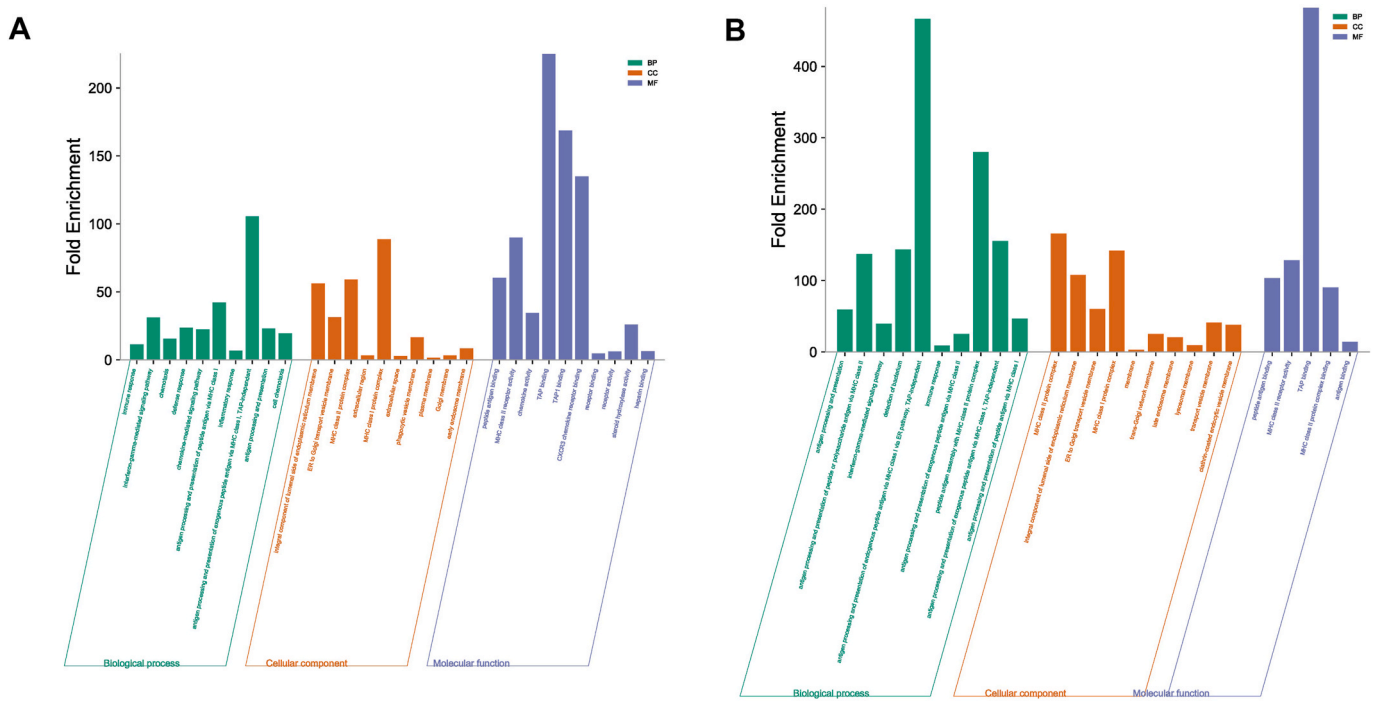


Fig. 5. Fig. 5a. The bar graphs of ontological analysis of Upregulated common gene in BD. Here biological process, molecular function, cellular component are shown.
Fig. 5b. The bar graphs of ontological analysis of Downregulated common gene in BD. Here biological process, molecular function, cellular component are shown.

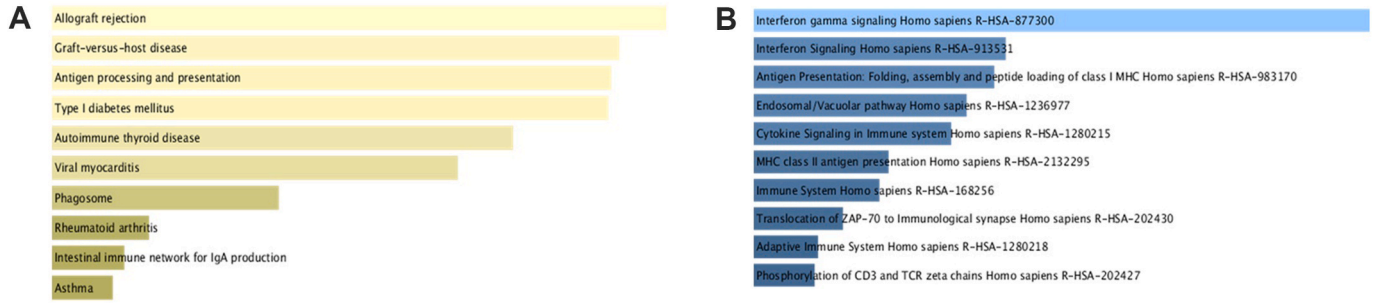


Fig. 6. The Enricher online tool generated bar graphs of pathway enrichment analysis of common DEGs in BD. Here, (a) KEGG pathway, and (b) Reactome pathway.

interaction analysis to see if any molecular signatures may be used as therapeutic targets or biomarkers for BD. The discovery of peripheral biomarkers may also offer information on the molecular basis of BD and allow for therapy monitoring. Transcriptomics analysis (through RNA-seq) is widely used to find possible biomarkers for a variety of disorders. RNA-sequencing (RNA-seq) is a process that requires massively parallel sequencing of the genome's transcribed region to provide a direct estimate of transcript abundance, with numbers proportionate to the target's absolute abundance [28]. Furthermore, One important purpose of our study is to determine how immunological markers in peripheral blood function at an individual level across distinct nosological groups. IgG1, Toxoplasma gondii IgG, and anti-cardiolipin antibodies are blood-based indicators required for the multi-domain model (ACA) [79]. Precision psychiatry with immunological and cognitive biomarkers: a multi-domain prediction for the diagnosis of bipolar disorder or schizophrenia using machine learning. Moreover, Blood biomarker data can be used to detect BD in patients who have just been diagnosed with MDD, reducing the risk of BD being misdiagnosed as MDD. Bioinformatics prediction and computer technology are now being used in various aspects of biological research. Since the gene expression profiles associated with psychosis, few comprehensive bioinformatics investigations for psychological disorders, which may differ significantly from those linked to other illnesses, have yet to be identified [3]. This research looked for DEGs in BD brain tissues, presenting a novel technique for identifying possible genes that could shed light on the disease's pathophysiology. The results revealed that two RNA-seq datasets found 122 common DEGs, with 82 being upregulated and 40 downregulated. Gene Ontology (GO) is a gene regulatory framework based on a generic theoretical model that simplifies the understanding of genes and their internal interactions. Over time, evolution achieved this by collecting biological knowledge about gene functions and regulation across a variety of ontological domains [80]. Interferon-gamma-mediated signaling pathways and cytokine-mediated signaling pathways are among the top GO terms for biological processes. Interferon-gamma (IFN-) is a type of cytokine that has a role in immune responses [81]. The key functions of IFN signaling are inflammation and cell-mediated immune responses [79]. Cytokines are proteins that help to regulate and orchestrate the immune response [82]. The top two GO keywords in the molecular function experiment are CXCR chemokine receptor binding and MHC class II protein complex binding. CXCR4 plays a vital role in the pathogenesis of psychiatric disorders, including MDD, BD, schizophrenia, and autism [83]. Recent research has linked

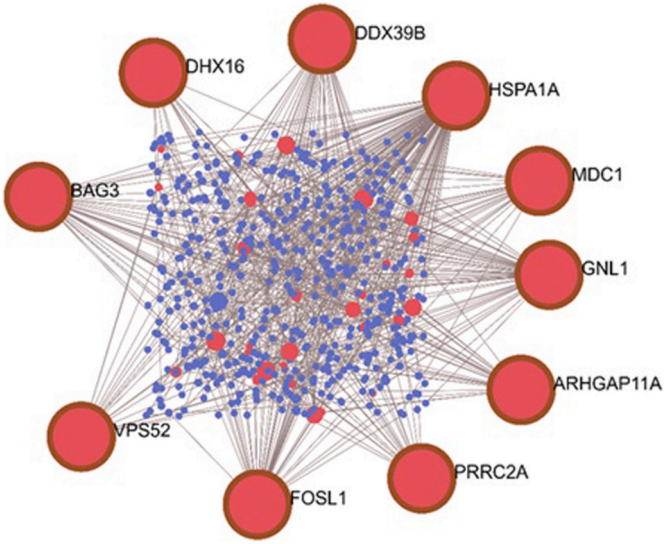


Fig. 7. DEGs are connected in a PPI network. DEGs are shown by nodes in the diagram, whereas interactions between two genes are represented by edges. The highlighted nodes are indicating the top 10 hub genes.

Table 3
The top ten PPI network HUB genes in terms of topological properties. (ranked by degree method).

Rank	Official Symbol of Hub genes	Degree	Full form	Regulation
1	HSPA1A	112	Heat Shock Protein Family A (Hsp70) Member 1A	Upregulated
2	GNL1	49	G Protein Nucleolar 1 (Putative)	Downregulated
3	FOSL1	48	FOS Like 1, AP-1 Transcription Factor Subunit	Upregulated
4	DDX39B	39	DExD-Box Helicase 39B	Downregulated
5	BAG3	33	BAG Cochaperone 3	Upregulated
6	ARHGAP11A	24	Rho GTPase Activating Protein 11A	Upregulated
7	MDC1	24	Mediator Of DNA Damage Checkpoint 1	Downregulated
8	PRRC2A	21	Proline Rich Coiled-Coil 2A	Downregulated
9	VPS52	19	VPS52 Subunit Of GARP Complex	Downregulated
10	DHX16	16	DEAH-Box Helicase 16	Downregulated

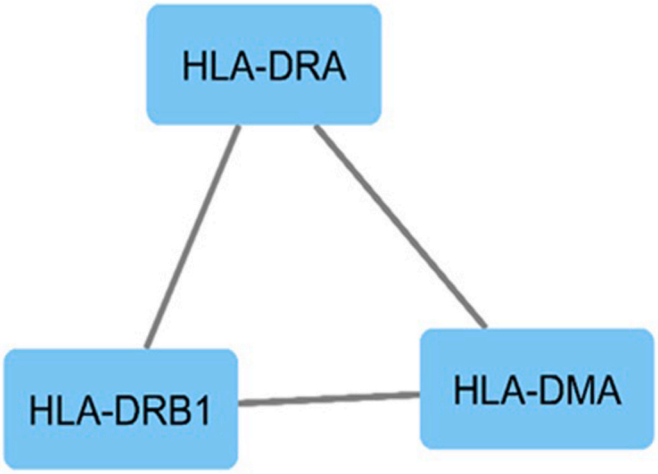


Fig. 8. The PPI network of DEGs in the BD's top one module. These nodes represent DEGs, while the edges represent interactions between two genes.

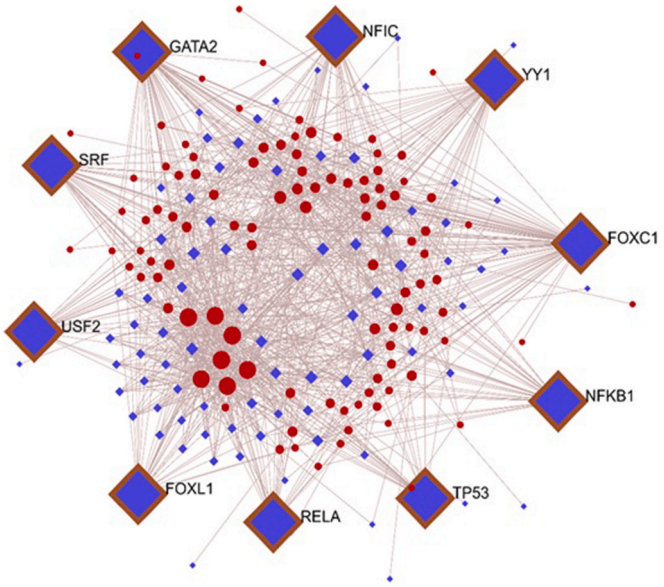


Fig. 9. The DEGs-TFs regulatory interaction network, as generated by NetworkAnalyst. The nodes in the diagram represent DEGs, whereas the edges reflect interactions between two genes. The highlighted nodes are indicating the top 10 TFs.

immune factors to the etiology of BD, and these factors are more specifically linked to different human major histocompatibility complex (MHC) regions. The top GO terms for cellular components are [84].

Pathway analysis has emerged as the preferred method for learning about the biology underlying differentially expressed genes and proteins because it decreases complexity while boosting the power of explanation [64]. The following are the top ten KEGG pathways: allograft rejection, graft-versus-host disease (GVHD), antigen processing and presentation, type 1 diabetes mellitus, autoimmune thyroid disease, viral myocarditis. Immune-related pathways, autoimmune disease networks (Type 1 diabetes, arthritis, graft-versus-host disease, and allograft rejection), and pathogen life cycle pathways were all altered by bipolar disorder [85]. The Reactome Knowledgebase systematically connects human proteins to their molecular functions, establishing a resource that may be used as a store for biological processes and a tool for detecting unanticipated functional correlations in data such as gene expression surveys [42].

A possible way for determining the disease's basic mechanisms is to evaluate the protein-protein interaction network. To find hub genes, we looked at the protein interactome network. These genes could be involved with BD.

The necessary regulatory biomolecules (TFs and miRNAs) have been discovered. As a result, biomolecule changes could provide crucial information on gene expression dysregulation in bipolar disorder. The regulatory network indicated ten transcription factors (FOXC1, GATA2, YY1, NFIC, SRF, NFKB1, FOXL1, RELA, USF2, TP53) that may be linked to BD and central cellular activities. FOXC1 is a molecule produced by brain pericytes during development necessary for fetal brain vascular development pericyte control [86]. GATA2 can operate independently of neuronal differentiation [87]. The knockdown of GATA2 resulted in a decrease in neuroglobin expression. The genes YY1 and FOXL1 have been linked to neurodegenerative disorders [88]. NFIC was discovered as a new locus in AD but not in BD [89]. SRF regulates the activation of the immediate early gene (IEG), linked to synaptic activity perception, learning, and memory. SRF is involved in processes involving actin cytoskeletal dynamics, such as developmental neuronal movement,

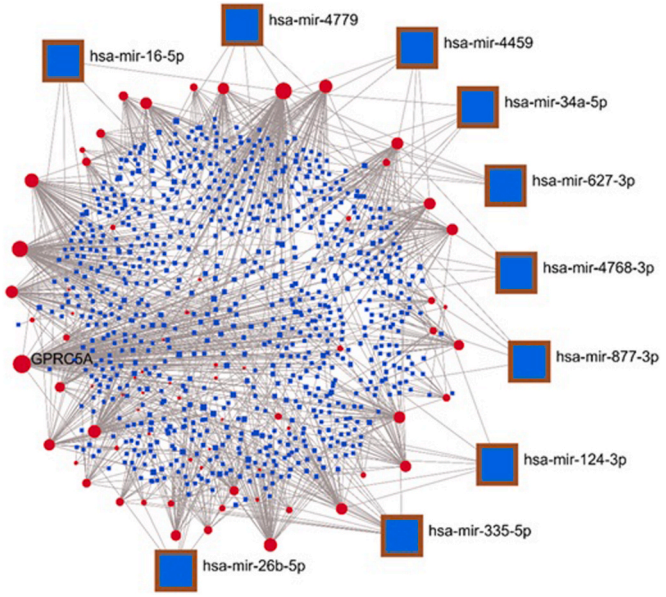


Fig. 10. The DEGs-miRNAs regulatory interaction network, as generated by NetworkAnalyst. The nodes in the diagram represent DEGs, whereas the edges reflect interactions between two genes. The highlighted nodes are indicating the top 10 miRNAs.

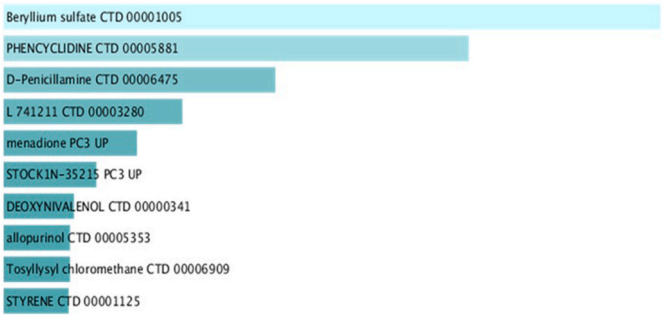


Fig. 11. The Enricher web tool displays candidate medicinal compounds as bar graphs.

neurite outgrowth and pathfinding, and synaptic targeting [90]. In a genome-wide association analysis in the Chinese Han population, NFKB1 was discovered to play a crucial role in the etiology of treatment-resistant schizophrenia [91]. YY1 and USF2 TF binding sites are overrepresented in neurodevelopmental and neuropsychiatric disorders [92]. Several acute traumas and neurological disorders cause neuronal death, and the TP53 gene is involved [93]. The RELA gene has genetic variations linked to the risk of schizophrenia [94].

Dysregulated miRNAs could be exploited as biomarkers and therapeutic targets in developing new BD therapeutics. The regulatory network indicated ten miRNAs (hsa-mir-335–5p, hsa-mir-124–3p, hsa-mir-4459, hsa-mir-627–3p, hsa-mir-34a5p, hsa-mir-26b-5p, hsa-mir-4768–3p, hsa-mir-4779, hsa-mir-877–3p, hsa-mir-16–5p). Parkinson's disease is associated with the hsa-mir-335–5p gene, but its role in BD is unknown [95]. The hsa-mir-124 is one of the most common miRNAs in the brain, and it is linked to things like neurogenesis and synaptic function control in mature neurons in both health and sickness, including cognitive impairment [96]. MiR-627–3p, which targets PTN, inhibits osteosarcoma cell proliferation and metastasis [97]. The hsa-mir-34a-5p biomarkers monitor drug treatment effects in children and adolescents with migraine pain [98]. These biomarkers were proposed to be related to BD for the first time and should be examined in future clinical studies.

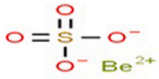
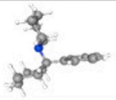
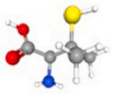
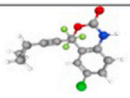
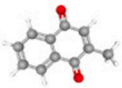
Name	P-value	Chemical Formula	Structure
Beryllium sulfate CTD 00001005	8.78×10^{-08}	BeSO4	
PHENCYCLIDINE CTD 00005881	9.35×10^{-07}	$C_{17}H_{25}N$	
D-Penicillamine CTD 00006475	1.02×10^{-05}	$C_5H_{11}NO_2S$	
L 741211 CTD 00003280	3.20×10^{-05}	$C_{14}H_9ClF_3NO_2$	
menadione PC3 UP	5.61×10^{-05}	$C_{11}H_8O_2$	

Fig. 12. The top five medication candidates for BD.

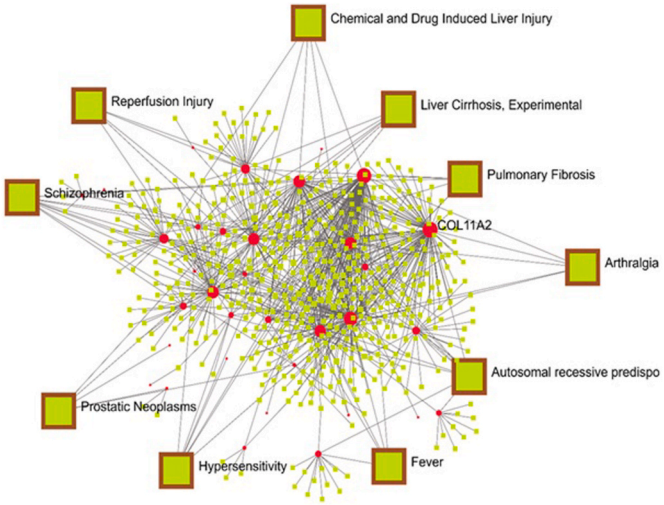


Fig. 13. Gene-disease association network obtained from NetworkAnalyst, The top ten gene-disease association network, is highlighted and reflects diseases linked to common DEGs. The nodes in the diagram represent DEGs, whereas the edges reflect interactions bet.

Table 4
Potential biomarkers validation through literature reviews.

SL No.	Hub gene	BD
1	HSPA1A	[23,67,68]
2	FOSL1	[69]
3	BAG3	[70,71]
4	PRRC2A	[72]
5	DHX16	[73]

Furthermore, we identified candidate drug molecules such as beryllium sulfate, PHENCYCLIDINE, D-Penicillamine, L741211 (Efavirenz), menadione, STOCK1N-35215, DEOXYNIVALENOL, allopurinol, Tosyllysyl chloromethane, STYRENE. Those can be treated for BD patients. Though Beryllium sulfate is toxic and may be related to lung

cancer, it can be a potent inhibitor of Myo-inositol monophosphatase, which has been isolated from rat brain, bovine brain, and a human neuroblastoma cell line [99]. Otherside, Phencyclidine is neurotoxic and has high abuse potential [100,101]. The etiology of mood disorder, bipolar disorder, schizophrenia has been linked to Phencyclidine [100,101]. D-penicillamine treatment can cause neurological aggravation or neuropsychiatric symptoms, such as cognitive difficulties, migraines, and psychosis [102,103]. D-penicillamine therapy has been shown to produce neurological aggravation or the emergence of neuropsychiatric symptoms, including movement abnormalities, seizures, and psychosis [102,103]. The noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP) is usually applied as a schizophrenia preclinical model in animals. Most of the studies on this model use approaches that look at behavior and brain abnormalities [100,101]. More research will be required. We anticipate that our procedure will help researchers detect early and direct appropriate treatment measures. Despite our best efforts, there are flaws in this study. The number of samples in the chosen datasets may not be enough to capture all of the key disease-associated genes needed to find the common DEGs. As a result, more research may be needed to properly examine the biological significance of the potential target candidates identified in this study.

5. Conclusion

Bioinformatics has made a considerable contribution to the genetic revolution. We used system biology and functional annotation approach to uncover important routes and biomolecules in bipolar disorder. We analyzed the transcriptomics of brain tissue to identify common DEGs from RNA-seq datasets. Following that, we used these common DEGs to analyze pathways for protein-protein interactions, transcription factors, and microRNAs. Using RNA-seq, 122 DEGs were discovered. DEG transcriptional and post-transcriptional regulators have been identified as TFs and miRNAs; as a result, we have discovered possible biomarker transcripts that are frequently dysregulated in BD brain tissues. We also suggested potential medications that target the biomarkers we discovered. To validate our findings as a predictive, diagnostic, and unique target to support the therapy of BD, we require a lab investigation. The bioinformatics technique utilized in this study could lead to new insights into potential targets involved in the risk of psychiatric diseases and drug-discovery programs that can help treat these conditions. Furthermore, numerous small compounds, such as Beryllium sulfate, could be a novel candidate medication for the treatment of BD. Overall, the current study identifies potential new targets for more personalized treatment of BD patients.

Author contributions

A. M. Humyra Islam, K. M. Salim Andalib, Sadia Afrin Bristy, and Md Habibur Rahman contribute to conception and design. A. M. Hunyra Islam performed the computational analyses and wrote the draft manuscript; Umama Khan, K. M. Salim Andalib, and Md. Shahadat Hossain helped prepare the tables and figures. Md. Abdul Awal, Md Habibur Rahman and Mohammad Ali Moni were involved in preparing the important intellectual content and critical revision; Md Habibur Rahman supervised the whole study. All authors approved the final version for submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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