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Transcriptomic Insights into Bipolar Disorder: Identifying Mood-States Master Regulators and Key Biological Processes

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Abstract

Aim

The current study aims to identify master regulator genes (MRGs) in blood samples from individuals with bipolar disorder (BD) during both acute episodes and remission periods.

Methods

In this study, we combined transcriptomic data with computational techniques to characterize disease-related molecular signatures through an in-depth analysis of differentially expressed genes (DEGs), proteins, and pathways. Five transcriptomic datasets, comprising a total of 165 blood samples from BD case-control studies, were analyzed to identify DEGs and construct transcriptional networks. Master regulator analysis (MRA) and gene set enrichment analysis (GSEA) were employed to identify key master regulator genes (MRGs) and associated biological processes across the different mood states of BD.

Results

The study identified 59 MRGs, with DMTF1 emerging as a common gene across all phases of BD, suggesting its potential as a universal biomarker. Additionally, phase-specific genes were identified: CLOCK and SETDB2 in depression, ZNF358 and ZNF787 in euthymia, and ELF4 in mania. These genes were linked to significant biological processes such as glucocorticoid receptor signaling in depression, inflammation in mania, and telomere protein regulation in euthymia.

Conclusions

Our findings provide valuable insights into the molecular mechanisms underlying BD and underscore the potential of MRGs as diagnostic biomarkers and therapeutic targets, particularly the role of DMTF1 in BD pathogenesis across different mood states. Despite these promising results, further research is needed, including studies with larger sample cohorts and biological validation through molecular biology methods.

1. Introduction

Bipolar disorder (BD) represents a complex and chronic psychiatric disorder characterized by mood fluctuations. The primary manifestations of BD are type I, involving manic, and type II, involving hypomanic episodes, along with depressive episodes (McIntyre et al. 2020). Epidemiological studies indicate that approximately 1.6% of the global population may experience BD in one of its forms during their lifetime (Merikangas et al. 2011). Despite its prevalence, the main challenges associated with BD are inherently linked to its complex nature and the difficulty of accurate clinical diagnosis. Distinguishing

episodes of bipolar depression from major depression (MDD) is particularly challenging due to (Phillips and Kupfer 2013) the symptomatic similarities between these conditions. Additionally, identifying lifetime hypomanic episodes can be difficult, as they are sometimes overlooked by patients and their caregivers.

In the face of these challenges, omics sciences, particularly transcriptomics, have emerged as valuable tools in the study of pathophysiology of BD (Sánchez-Baizán et al. 2022). This approach, rooted in molecular biology and bioinformatics, has played a significant role in examining transcripts. For instance, messenger RNAs (mRNAs) produced by an organism at specific times or under particular conditions offer valuable insights into various diseases, including the distinct phases and subtypes of BD. This enables the monitoring of disease progression and provides personalized treatment options through the identification of biomarkers (Gandal et al. 2018).

Master regulatory genes (MRGs) which function as transcription factors or regulators of gene expression, emerge as promising biomarker candidates in psychiatric disorders (Ohno 1978). Belonging to gene families or signaling pathways, they play a prominent role in regulating cellular cascades and represent the highest level of regulatory hierarchy (Cai et al. 2020). Transcriptomic approach, combined with sophisticated statistical methods such as gene set enrichment analysis (GSEA) can effectively identify genes enriched in samples from different groups, thereby providing valuable insights into the most relevant biological processes and their interrelationships (Subramanian et al. 2005). Therefore, exploring transcriptomic tools aims to deepen the understanding of MRGs and their potential as biomarkers candidates could improve the diagnostic accuracy of BD and enrich our knowledge of the underlying disease mechanisms. The current study aims to identify MRGs in blood sample form subjects with bipolar disorders during acute episodes and remission periods.

2. Material and methods

2.1 Data Acquisition

The rationale behind selecting datasets for studying regulatory genes in BD is fundamental. We chose to acquire data exclusively from patients with BD stratified by phase, to enable a comprehensive analysis of MGRs at each specific mood state of BD. Furthermore, we decided to use only microarrays as the transcriptomic technique was motivated to minimize technical variability. By focusing on a single technique, we reduce potential sources of variation, thereby enhancing the reliability and comparability of the results. This careful selection of datasets and acquisition techniques establishes the foundation for a robust investigation into regulatory genes in the context of BD.

A meticulous data acquisition and differential expression analysis was undertaken to elucidate the regulatory landscape of MGR in BD. We conducted a search on the Gene Expression Omnibus (GEO) repository using predefined criteria: ("bipolar disorder"[MeSH Terms] OR bipolar disorder[All Fields]) AND ("blood"[Subheading] OR "blood"[MeSH Terms] OR blood[All Fields]). Further refinement involved applying

filters for "homo sapiens" and "expression profiling by array," resulting in the identification of 25 databases. The inclusion criteria consisted of a) case-control studies in which the mood state of BD was previously characterized; b) transcription factors assessed by microarray analysis, and c) studies conducted on human blood samples.

Five microarray transcriptomics datasets representing distinct phases of BD (euthymia, mania, depression) were selected from the GEO repository: [GSE121963, GSE46416, GSE45484, GSE39653, GSE23848] (Fig. 1). The datasets were downloaded using the GEO query tool and subjected to a rigorous filtering process. Batch correction was performed using the virtualArrayComBat function (Davis and Meltzer 2007; Johnson et al. 2007; Heider and Alt 2013), resulting in 165 samples (Table 1).

Table 1
Sample Characterization.

GEO ID	PMID	Mood state	Age	Gender	n	Tissue	Medication
GSE121963	31118907	Mania x Euthymia	45,2 ± 9,9	50% male	6	Whole blood	No individual information
GSE46416	25136889	Mania x Euthymia	48,3 ± 12,0	100% male	11	Whole blood	Antipsychotics, Mood stabilizers, Anticonvulsants, antidepressants, Benzodiazepines
GSE45484	23670706	Depression	39,45 ± 12,2	31,6% male	60	Whole blood	Mood stabilizers
GSE39653	23064081	Depression	35 ± 10,0	32% male	29	PBMC	No medication
GSE23848	21176028	Depression	38.4 ± 9,5	30% male	20	Whole blood	Antipsychotic, Mood stabilizers, Anticonvulsants

GEO ID, Gene Expression Omnibus ID; PMID, PubMed ID; Mood state, indicates the mood state of the subjects (e.g., Mania, Euthymia, Depression). Age, mean age of the subjects, presented as mean ± standard deviation, Gender, percentage of male subjects in the sample. N, total sample size; tissue, type of tissue used for analysis; Medication, medication status of the subjects (types of medications administered).

2.2 Differential Expression Analysis

Differential expression analyses were performed utilizing the *limma package*, employing multiple linear models and moderated t-statistics with empirical Bayes moderation (Johnson et al. 2007). Genes with an unadjusted p-value < 0.05 and a fold change exceeding 15% were deemed differentially expressed genes (DEGs). To establish a baseline, healthy human blood samples were obtained from two large microarray datasets [GSE48348 and GSE99039] obtained from the GEO repository. These datasets, comprising 967 healthy blood samples, were combined and processed using the *GEOquery* and *virtualArray* packages, including a batch correction to mitigate potential batch effects (Davis and Meltzer 2007; Johnson et al. 2007; Heider and Alt 2013). This comprehensive dataset was then employed for

subsequent transcriptional network inference, facilitating a deeper understanding of the regulatory dynamics in BD.

2.3 Transcriptomic Reference Networks and Reverse Engineering

Transcriptomic reference networks provide a comprehensive view of gene interactions and regulatory dynamics, shedding light on the underlying molecular mechanisms. The decision to construct transcriptomic reference networks in this study stems from their ability to capture intricate gene relationships and identify key regulatory elements. To ensure the reliability of our analyses, a series of meticulous data preprocessing steps were implemented. These included normalization techniques to account for potential biases and variations in gene expression across different datasets.

A transcriptional reference network (TN) centered on transcription factors (TFs) and their predicted target genes were inferred using a large cohort of healthy blood samples and merged as described in “Data Acquisition.” Herein, “regulatory unit” or “regulon” describes the groups of inferred genes and their associated TFs. The construction of these networks utilized the RTN (v2.16.1) package and the Algorithm for the Reconstruction of Accurate Cellular Networks (ARACNe) method, as described by Fletcher et al. (2013) and Margolin et al. (2006). This approach allowed us to infer gene interactions based on co-expression patterns, establishing a foundation for identifying MGR within the BD context.

In summary, the regulatory structure of the network is derived by mapping significant associations between known TFs and all potential targets. A curated list of genes (Lambert et al. 2018) was used to annotate TF eligibility for TN inference input. To create a consensus bootstrap network, a permutation step eliminates the interactions below a minimum MI threshold, and unstable interactions are additionally removed by bootstrap. Finally, the data processing inequality algorithm is applied with null tolerance to eliminate interactions likely to be mediated by a third TF. The reference blood TN was built using the package’s default number of 5000 permutations and 100 bootstraps (p-value < 0.001).

2.4 Master Regulator Analysis and Gene Set Enrichment Analysis

To identify key regulators governing gene expression patterns, we conducted a master regulator analysis (MRA) following the approach outlined by Carro and colleagues (Carro et al. 2010). MRA is a crucial step in unraveling the intricate regulatory networks of master regulatory genes.

For each regulatory unit within the constructed blood transcriptional network (TN), the algorithm employed computes statistical overrepresentation using a modified Fisher's exact test. This statistical analysis aims to discern whether the genes obtained from differential expression analyses exhibit a significant enrichment (unadjusted p-value < 0.05 and a fold change over 15%) within the regulatory unit. The choice of a modified Fisher's exact test was driven by its ability to robustly capture statistically

significant associations within our study on master regulatory genes. These associations are pivotal in unveiling potential regulators that control gene expression dynamics.

Additionally, we conducted a one-tailed Gene Set Enrichment Analysis (GSEA) to assess whether members of specific gene sets are associated with phenotypic class distinctions (Subramanian et al. 2005). This complementary approach further enhances our understanding of the functional relevance of the identified master regulators.

2.5 Functional Significance Analysis using Enrichr

To unravel the functional significance of the identified genes associated with master regulatory processes, we leveraged the Enrichr software application (Chen et al. 2013). Enrichr is recognized for its capabilities in providing comprehensive functional annotations and has been widely utilized in similar studies.

The combination of MRA and GSEA results allowed us to pinpoint biological processes linked to distinct mood states, including depression, euthymia, and mania. Subsequently, the generated bar chart from the Enrichr analysis was strategically sorted based on the ranking of combined scores. The combined score is calculated by taking the logarithm of the p-value derived from the Fisher exact test and multiplying it by the z-score, representing the deviation from the expected rank. This approach provides a robust statistical basis for ranking biological processes, with terms possessing higher combined scores considered most relevant in the analysis.

The decision to sort the bar chart based on the ranking of combined scores was driven by highlighting and prioritizing the most biologically significant terms associated with the identified MGR. This sorting strategy contributes to a clearer interpretation of the functional relevance of these genes in the context of depression, euthymia, and mania.

3. Results

3.1 Master Regulator Analysis

The MRA is an approach that aims to identify the TFs acting as MRGs responsible for changes in gene expression between different conditions. In our study, the MRA identified 59 potential (Supplementary Table 1) MRGs modulating the regulatory units enriched with genes altered in BD, as shown in Fig. 2. We observed specific MRGs in patients during depressive and euthymic phases, but no exclusive MRGs were identified during mania. Additionally, we found 12 MRGs that were in common between euthymia and depression and two MRGs that were common between depression and mania. Interestingly, we found a common MRG, DMTF1, shared across all three phases of BD. Based on this discovery, we focused on DMTF1 to assess its potential as a disease marker, given its consistent prominence in BD across all phases.

3.2 Gene Set Enrichment Analysis

One-tailed GSEA evaluates whether a predefined set of genes, based on biological information, is enriched among DEGs across various conditions. Using GSEA, we identified 134 enriched genes (Supplementary Table 2), of which 2 (CLOCK and SETDB2), 3 (ZNF358, ZNF653, and ZNF787), and 4 (ELF4, ZNF467, ZNF512, and ZNF91) were found in depression, euthymia, and mania, respectively. The intersection between BD phases led to the identification of 31 genes, as shown in Fig. 3. Based on this analysis, we explored the unique MRGs to highlight them as phase markers of BD.

3.3 Biological Processes

To discover biological processes relevant to BD, we employed the results from MRA and GSEA for the different phases of the diseases. Through the Enrichr software, we identified the following prominent biological processes associated with depression, euthymia, and mania: negative regulation of the glucocorticoid receptor signaling pathway, establishment of protein localization to telomeres, and retrotransposon silencing. Notably, regulation of the apoptotic process in smooth muscle cells was identified as a secondary process in depression, and this is connected to the DMTF1 gene. Additionally, in euthymia, the biological processes involve the regulation of DNA damage response and signal transduction by the p53 class mediator, both of which are related to the DMTF1 gene. In contrast, mania revealed a significant portion of its processes related to inflammation (Fig. 4).

4. Discussion

The current investigation employed a transcriptomic approach and systems biology to delineate transcription factors functioning as MRG in BD. *In silico* analysis was conducted utilizing microarray data derived from blood samples of individuals with BD during acute episodes and remission periods. The MRA allows for identifying key regulators that exert control over many genes in a gene network, while GSEA helps identify sets of genes that are coordinately involved in specific biological processes. The integration of these two tools facilitated the unveiling of crucial biological patterns integral to the disease. Through MRA, we identified 59 candidate MRGs associated with BD, with DMTF1 emerging as the sole gene in all three mood states. Employing GSEA, we uncovered 134 enriched genes, with 2, 3, and 4 genes exclusively identified in depression, euthymia, and mania. Utilizing the Enrichr tool, we further delineated the most prominent biological processes characterizing each phase of BD from these data. Our findings may contribute to advance our understanding of BD and pave the way for improved diagnostic strategies and personalized treatment options.

DMTF1: A Gene Connects the Dots Across the Phases of BD

Analyzing the specificity of connections in each phase of BD provides a valuable perspective for understanding the molecular mechanisms underlying this complex psychiatric disorder. Given the presence of the DMTF1 gene in all three phases of BD, we will seek to understand its association with

the disorder, considering the specificity of its connections in each phase. DMTF1, a tumor suppressor, triggers the transcription of ARF, initiating the ARF-p53 pathway known for promoting cellular senescence or apoptosis (Fry and Inoue, 2019). When activated, p53 triggers cellular responses, such as temporary cell cycle arrest for DNA repair, senescence, or apoptosis (Carrasco-Garcia et al. 2017). Interestingly, a polymorphism in the human p53 tumor suppressor gene (TP53) was associated with susceptibility to BD in the Chinese Han population, suggesting that genetic variations in this gene may influence predisposition to BD (Yang et al. 2019). The establishment of protein localization to telomere and retrotransposon silencing were identified biological processes involved in the etiology of BD. Considering that DMTF-1 helps maintain genomic stability by regulating genes involved in DNA repair and genome integrity, it is possible to speculate that DMTF-1 could influence retrotransposon activity and telomere maintenance.

Studies indicate that abnormal neuronal apoptosis is involved in neurophysiological processes associated with BD pathogenesis. For example, a post-mortem electron microscopy study revealed signs of apoptosis in oligodendrocytes of the frontal lobe in BD patients during depression. These signs included cell reduction, smaller nuclear size, and nuclei condensation (Uranova et al. 2001). In the lymphocytes of BD patients, a decrease in the expression of the anti-apoptotic factor HSP70 and reduced levels of the pro-apoptotic protein BAX in the cytosolic fraction were observed, indicating its translocation to the mitochondria to induce apoptosis (Bei et al. 2009). Pietruczuk et al. also found increased apoptosis in the lymphocytes of BD patients, especially during depression, compared to controls. In an in vitro experiment, lithium and valproate protected patients' lymphocytes from apoptosis (Pietruczuk et al. 2018).

It is reasonable to speculate that an imbalance between cell cycle arrest and apoptosis is a significant mechanism underlying BD. Such an imbalance may impair cellular responses to stress, rendering cells more susceptible when exposed to stressful situations. This imbalance could also contribute to increased oxidative stress, mitochondrial dysfunction, inflammation, and DNA damage, commonly observed in the brains and peripheral tissues of individuals with BD.

CLOCK and SETDB2 as Potential Phase Markers for Bipolar Depression: Unraveling the Interplay with Glucocorticoids (GC)

In our study, dysregulation of glucocorticoid receptor signaling emerges as a prominent biological process during the depressive phase of BD. Glucocorticoids, steroid hormones released by the adrenal gland, are vital for regulating stress response, metabolism and immune function and possess potent anti-inflammatory properties (Wiley et al. 2016). Elevated glucocorticoid levels in depression patients suggest impaired feedback regulation of the hypothalamic-pituitary-adrenal (HPA) axis, potentially due to altered glucocorticoid receptor (GR) function (Anacker et al. 2011). Animal models have also demonstrated how chronic stress and high glucocorticoid exposure can damage the hippocampus, leading to a self-perpetuating cycle of elevated glucocorticoid levels and contributing to behavior that mimic depression (Czéh and Lucassen 2007). Notably, a clinical study showed a substantial increase in

glucocorticoid receptor (GR) levels within four weeks of antidepressant treatment, coinciding with improvements in clinical symptoms and Hamilton Depression Rating Scale (HDRS) scores (Pariante and Miller 2001). This finding highlights GR as a promising molecular target for antidepressant therapy.

Our analysis revealed a noteworthy enrichment of two genes, namely CLOCK and SETDB2, specifically heightened during the depressive phase of BD. This finding gains even more relevance considering the intrinsic connection of these genes with the biological process of GR signaling dysregulation. Glucocorticoids play a crucial role in modulating the circadian rhythm of immune responses, establishing a complex interaction mediated, in part, by the direct interaction between GR and the histone acetyltransferase CLOCK. The connection between the CLOCK gene and GR suggests an association between epigenetic processes and circadian rhythm-related gene expression (Nader et al. 2009).

The CLOCK gene is essential for maintaining a healthy and functional circadian rhythm, and its alterations or dysfunctions may be associated with sleep disorders and mood disorders (Schuch et al. 2018). The literature indicates that the CLOCK protein in the hippocampus is crucial for the long-term development of depressive-like behavior caused by chronic unpredictable stress. Moreover, another study proposed that polymorphisms in the CLOCK gene may contribute to the recurrence of BD episodes in individuals experiencing a depressive mood (Benedetti et al. 2003). Also, an interesting study revealed that variations in the CLOCK gene can influence the disparity between how individuals with BD perceive their depression and how healthcare professionals objectively assess it (Suzuki et al. 2017). Therefore, it is reasonable to infer that genetic variations in the CLOCK gene may be linked to the biological basis of depression. These genetic variations can impact the body's "biological clock," affecting sleep patterns, mood, and emotional well-being. Taken together, all these results suggest that circadian rhythms stability works as a therapeutic target for the management of depressive episodes (Jiang et al. 2013).

Meanwhile, the association between SETDB2 and GR signaling becomes evident in the modulation of lipogenesis. Activation of GR leads to elevated expression of Insig2a, thereby inhibiting sterol regulatory element binding proteins (SREBPs) (Yabe et al. 2002). SETDB2 expression is potentiated during fasting or dexamethasone treatment, indicating positive regulation of its coactivator by GR. GC-mediated suppression of SREBP processing and increased lipogenesis occur when SETDB2 is silenced. The glucocorticoid-responsive interaction between SETDB2 and GR provides a mechanistic insight into the mode of action of GR in transcriptional regulation in the liver, highlighting SETDB2 as a potential therapeutic target for modulating glucocorticoid action in metabolic diseases associated with altered glucocorticoid sensitivity (Roqueta-Rivera et al. 2016).

SETDB2 is an enzyme belonging to the histone methyltransferase family that plays an essential role in epigenetic modifications (Tachibana et al. 2015). Despite limited evidence in psychiatry or neurology, a preclinical study showed that SETDB1 regulates mood-related behaviors and the expression of NMDA receptor subunits, specifically the NR2B subunit, suggesting the promising role of this gene in neuronal communication (Jiang et al. 2010). Another study highlights the role of the enzyme SETDB1 in regulating

the development of Htr3a-positive interneurons and how this may be related to mood-related behaviors (Li et al. 2023). Moreover, a study investigates the function of SETDB1 as a histone methyltransferase, impacting the Toll-like receptor 4 (TLR4)-mediated inflammatory response in macrophages. This highlights the significance of SETDB1 in immune response and inflammation, with potential implications for mood-related condition (Hachiya et al. 2016). Given the evidence of the SETDB family's presence in behavioral experiments, it is plausible to suggest that, in conjunction with other genes, it could serve as a potential mood state marker for bipolar depression.

Inflammation Dynamics in Bipolar Mania: The Interplay of IL-1β, ZNFs, and ELF4 in Biological Processes

Among biological processes, inflammation was highlighted in manic state, which is consistent with the existing literature (Kim et al. 2007). Interestingly, our study highlights the role of interleukin 1-beta (IL1β) in biological processes associated with the manic phase in BD. IL-1β is a pro-inflammatory cytokine that plays a fundamental role in the body's immune and inflammatory response. Epidemiological evidence reveals that there is an increase in IL-1β levels in the peripheral circulation and cerebrospinal fluid in patients diagnosed with BD (Söderlund et al. 2011). Multiple therapeutic strategies employed in the treatment of mood disorders have been associated with the ability to influence IL-1β levels. Furthermore, studies performed in preclinical models have demonstrated that administration of IL-1β has the potential to alter behavioral and neurochemical responses that are considered pertinent to mood functioning in humans (Koo and Duman 2009). Literature also compared patients with recent manic or hypomanic episodes (at least 1 in the year before cerebrospinal fluid collection) and patients without these episodes. Patients with recent episodes had higher IL-1β levels than those without. These results suggest the possibility that the concentration of this cytokine may vary across different states of BD (Söderlund et al. 2011). However, there is a divergence in the results regarding IL-1β since other studies indicate that the levels of this cytokine are increased during the depressive phase and decreased in the manic phase of BD (Ortiz-Domínguez et al. 2007). This complexity highlights the need for additional investigations to better understand the dynamics of IL-1β in the phases of BD, especially manic. Furthermore, the elevation of pro-inflammatory cytokines has been associated with increased retrotransposon activity in various tissues. Retrotransposons are typically kept inactive by the cell's defense mechanisms, such as DNA methylation, histone modifications, and small RNA pathways. However, they can become activated and move within the genome under certain conditions, such as cellular stress, inflammation, or epigenetic dysregulation. The transposon activity may influence the process of aging and age-related diseases.

The manic phase of BD revealed ELF4 as an enriched gene. This gene plays a role in various biological processes, including immune and inflammatory responses (Suico et al. 2016). Hyperinflammatory responses were identified in macrophages with mutations in ELF4, both in human cells from patients and in mouse models. In mice, ELF4 stimulated the expression of genes with anti-inflammatory properties while restraining the activation of inflammation-related genes. Inhibiting the Trem1 gene, which plays an inflammatory role, successfully reversed inflammation and intestinal pathology in mice carrying ELF4 variants derived from patients. These findings establish ELF4 as a key regulator in the

modulation of inflammation at the transcriptional level (Tyler et al. 2021). Another study described that loss of function of ELF4 leads to compromised type I interferon response in ELF4-deficient mice after viral and bacterial infections. Additionally, there was a significant increase in the production of pro-inflammatory cytokines such as interleukin-6 (IL-6), IL-1b, and tumor necrosis factor alpha (TNF-α) in patients and mutant mice (Sun et al. 2022). Additionally, investigators examined the impact of ELF4 on inflammatory cytokines following ischemia/reperfusion (I/R) injury. The results revealed a substantial increase in inflammatory cytokines such as IL-6, TNF-α, IL-18, and IL-1β during I/R, with an even more pronounced inflammatory response observed in the absence of ELF4. Moreover, I/R injury led to elevated levels of proteins associated with pyroptosis within renal tissues, with this effect exacerbated by ELF4 deficiency. Manipulation of ELF4 in cells subjected to ischemia/reperfusion conditions resulted in significant changes in the levels of inflammation-related proteins, either increasing or decreasing them markedly (Li et al. 2023).

Besides the family mentioned above, the manic phase also presented genes from the zinc finger protein family enriched. Interestingly, this same family of proteins was also observed in euthymic patients. This similarity may be related to mood-stabilizing medications used in both manic and euthymic patients. Zinc finger proteins (ZNFs) are highly diverse in terms of molecular functions and represent one of the most prolific protein groups. ZNFs can interact with various molecules, including DNA, RNA, poly-ADP-ribose (PAR), and other proteins (Cassandri et al. 2017). Recently, Genome-Wide Association Studies (GWAS) have provided the basis for the hypothesis that ZNFs might be linked to susceptibility to psychiatric disorders, including schizophrenia, bipolar disorder, and major depressive disorder (Amare et al. 2020) as well as the mechanism of action of psychotropic drugs. For instance, a study revealed that some ZNFs genes are affected by the lithium administration, specially in patients who are responders to lithium treatment. ZNF493 seems to modulate lithium mechanisms, presenting itself as a potential target for lithium responsiveness (Pisanu et al. 2018). Despite encouraging, these findings still require additional studies in independent samples to understand better the role of ZNFs on psychiatric disorders (Squassina et al. 2019).

Biomarkers of Aging: Investigating Telomere Dynamics as a Window into BD during Euthymia

Our results showed, during the euthymic phase, the establishing protein localization at the telomere is a prominent biological process. The telomeres are specialized structures composed of repetitive DNA sequences and associated proteins residing at the ends of chromosomes, which play a crucial role in maintaining genomic stability (Rossiello et al. 2022). Their primary function is to safeguard the genetic material from deleterious events, such as DNA degradation, non-homologous recombination, improper repair, and aberrant chromosomal fusion (Zhu et al. 2019). These dynamic structures are essential in maintaining genome integrity throughout cell divisions (Cherif et al. 2023). As a common cellular process, telomeres shorten with age, and the progressive shortening of telomeres leads to senescence and/or apoptosis. Telomere length plays an essential role in the pathophysiology of BD, demonstrating to be a link between BD and medical comorbidities. Indeed, telomere shortening may explain, in part, the life expectancy reduction in subjects with BD.

Limitations

Recognizing the constraints in our research is crucial. It encompasses sample heterogeneity and the limitations inherent in our algorithms. Some studies do not provide complete clinical information, including mood state, duration of illness, comorbidities, or pharmacotherapy, all of which may influence the results. However, the selection of datasets was performed to minimize sample differences. Our study used only peripheral transcriptomics data, which could restrict our samples' overall representation and the brain's complexity. Bioinformatic techniques and algorithms constantly evolve, and choosing the right tool for a specific analysis can be challenging. Additionally, some tools may have accuracy or processing capability limitations. Also, the lack of standardization in data formats and analysis methods can hinder integrating results from different studies. This lack of standardization can lead to inconsistencies and difficulties in data comparison.

Conclusion

This study employed a transcriptomic approach coupled with bioinformatics revealed distinct transcriptomic signatures associated with depression, euthymia, and mania, thereby highlighting the intricate genetic alterations characterizing each mood state in BD. The most prominent processes involved in BD were negative regulation of the glucocorticoid receptor signaling pathway, immune system and telomere dynamics. Moreover, MRA Identified the consistent presence of DMTF1 across all mood states of BD, suggesting its potential as a promising trait biomarker candidate of the disorder. Understanding these transcriptomic patterns has significant clinical implications, suggesting the potential identification of biomarkers and therapeutic targets specific to each phase of BD. However, it is crucial to recognize the limitations of this study and the ongoing need for in-depth investigations to fully elucidate the regulatory network involved in BD and translate these findings into practical advancements in clinical practice.

Abbreviations

MRGs
master regulator genes
BD
bipolar disorder
DEGs
differentially expressed genes
MRA
Master regulator analysis
GSEA
gene set enrichment analysis
MDD
major depression

mRNAs
messenger RNAs
GEO
Gene Expression Omnibus
TN
transcriptional reference network
TFs
Transcription factors
ARACNe
algorithm for the Reconstruction of Accurate Cellular Networks
TP53
human p53 tumor suppressor gene
GR
glucocorticoid receptor
Hamilton Depression Rating Scale
HDRS
SREBPs
sterol regulatory element binding proteins
GC
Glucocorticoids
TLR4
Toll-like receptor 4
IL1β
interleukin 1-beta
IL-6
interleukin 6
TNF-α
tumor necrosis factor alpha
I/R
ischemia/reperfusion injury
ZNFs
Zinc finger proteins
PAR
poly-ADP-ribose
GWAS
Genome-Wide Association Studies

Declarations

Competing interests

No potential conflict of interest relevant to this article was reported.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

All authors gave consent for publication in this Journal.

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Author Contribution

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conceptualization, ARR, MAB, and PRZ; Methodology, PRZ and MAB. Formal Analysis, MAB; Writing - Original Draft, PRZ, PHR, TS, GM, GHH, and QC; Writing - Review & Editing, ARR and FK.

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Data Availability

The microarray transcriptomics datasets analyzed in this study were retrieved from the Gene Expression Omnibus (GEO) repository using predefined criteria described in the Methods section of the manuscript. The following datasets representing distinct phases of bipolar disorder were included: GSE121963,

GSE46416, GSE45484, GSE39653, and GSE23848, and are publicly available at the GEO database: https://www.ncbi.nlm.nih.gov/geo/.

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Figures

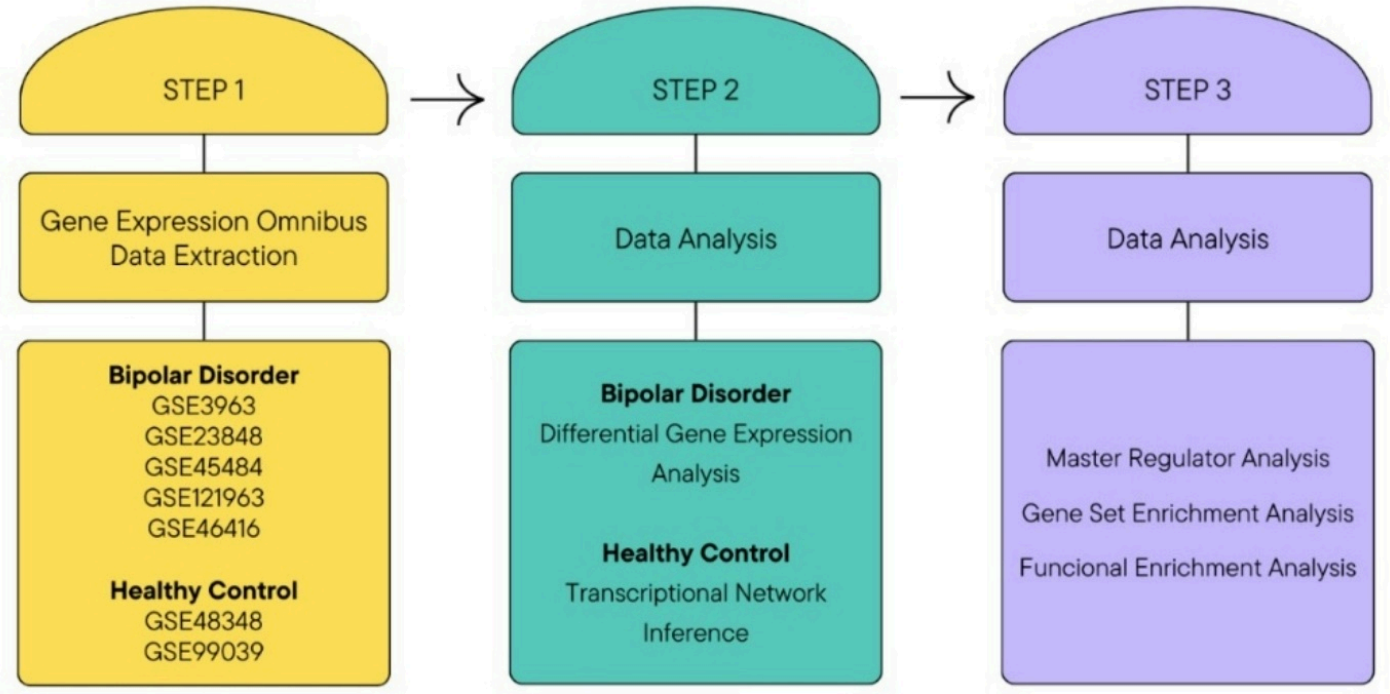


Figure 1

Methodology flowchart. Bipolar disorder and healthy control data were obtained from the Gene Expression Omnibus Database. Healthy human blood gene expression data was used to reconstruct a normal blood regulatory network centered on transcription factors. Differential expression genes were computed from controls and each phase of bipolar disorder patients' data. Master Regulator Analysis (MRA) and Gene Set Enrichment Analysis (GSEA) were executed by differential expression genes. Enrichr was used to conduct functional enrichment analysis.

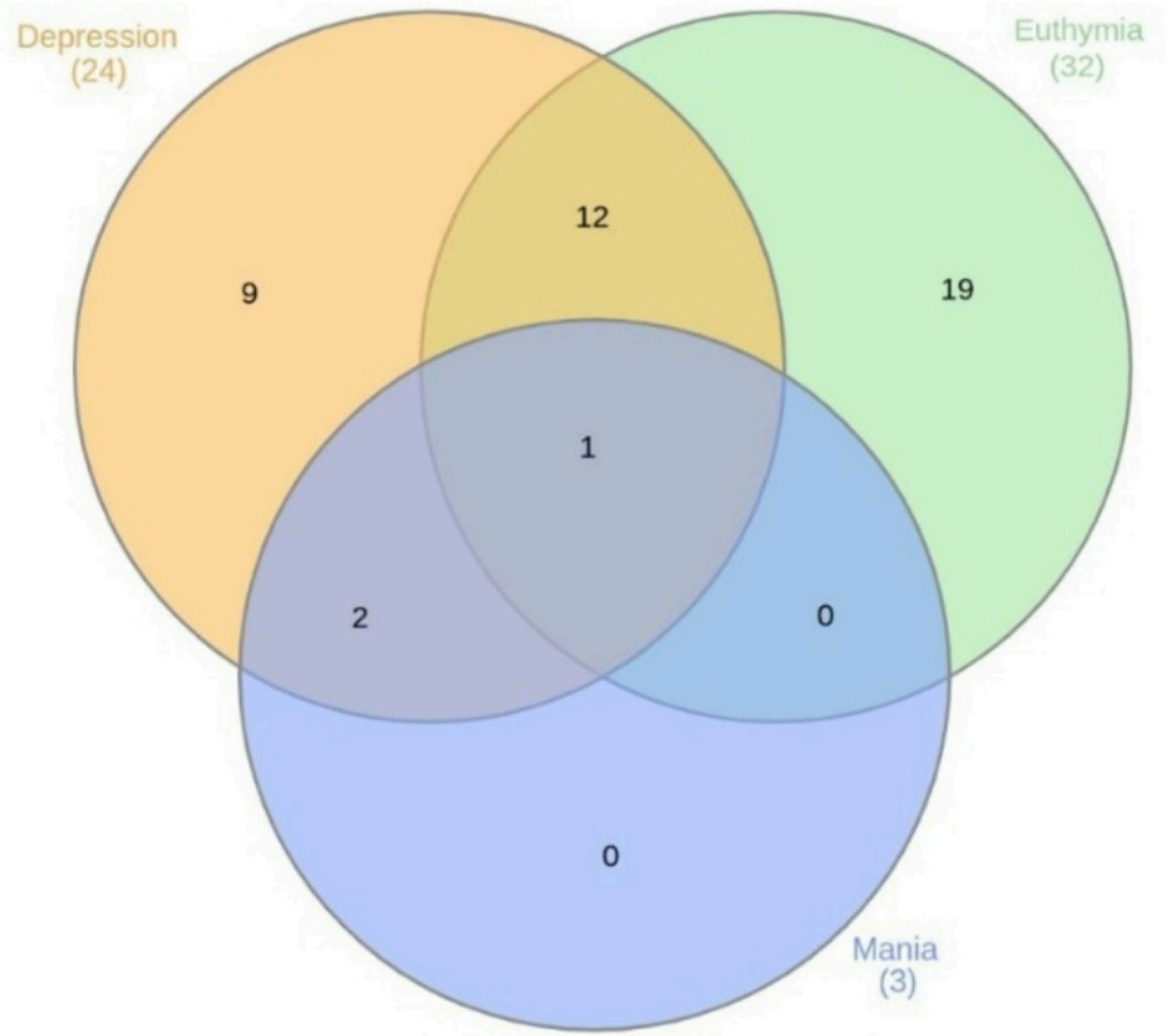


Figure 2

Venn Diagram for Master Regulator Analysis results.

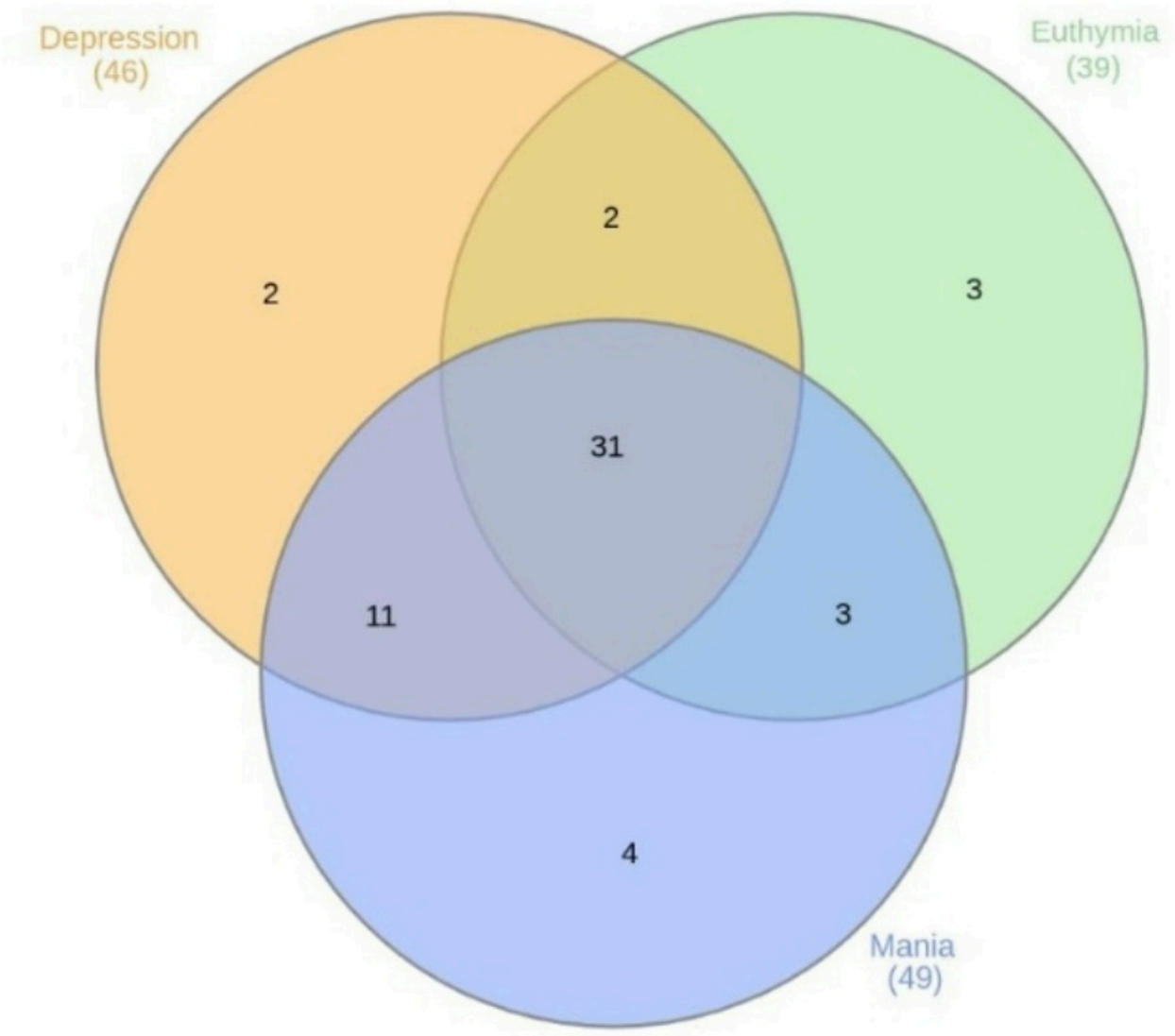


Figure 3

Venn Diagram for Gene Set Enrichment Analysis results.

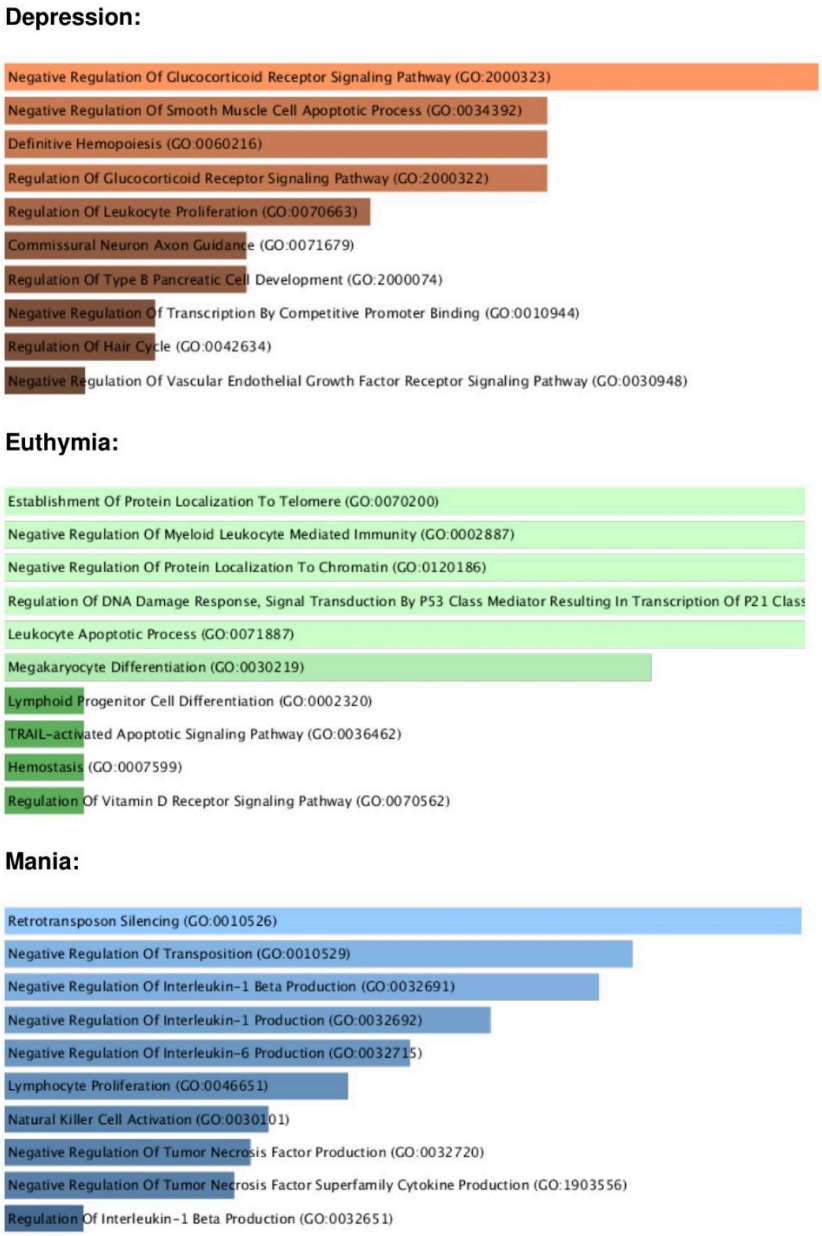


Figure 4

Biological processes found by Enrichr software. The bar chart generated by Enrichr was sorted based on the ranking of combined scores.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supp1MRAresults11.xlsx
- Supp2GSEAresults11.xlsx