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In Silico Identification of Key Genes and Pathways Associated with Bipolar Disorder Using GWAS

A research submitted as a fulfilment of requirements of a Master's degree in Bioinformatics

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Abstract

Bipolar disorder **(**BD**)** is a chronic and recurrent disorder that affects more than (1%) of the global population. The most prevalent age for the onset of symptoms is 20 years old; early-onset is associated with a worse prognosis. It is a leading cause of disability in young people as it can lead to cognitive and functional impairment and increased mortality, particularly from suicide and cardiovascular disease.

Our analysis drew upon Genome-Wide Association Studies (GWAS) from the Psychiatric Genomic Consortium (PGC) and GWAS Catalog for BD patients. Through the analysis; 118 genomic risk loci and 539 genes were mapped. By utilization of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, a deeper understanding of the underlying biological processes and crucial pathways related to BD was attained. As a result, a comprehensive protein-protein interaction (PPI) network was established, revealing 16 central hub genes and two notable modules.

Using the Comparative Toxicogenomics Database (CTD), we performed *in-silico* validation of the hub genes. Our findings from functional enrichment analysis highlighted the crucial functions of these key genes in biological processes such as antigen processing and presentation and regulation of T-cell mediated immunity. Additionally, we identified 762 microRNAs and 28 transcription factors that target these hub genes, further supporting their significance in BD disorder.

By conducting a thorough bioinformatics analysis, we have gained insights into the underlying mechanisms of BD, identifying potential biomarkers for clinical treatment, and uncovering drug targets. These findings greatly enhance our understanding of BD and show potential for improving diagnosis and treatment methods in the future.

Keywords: bipolar disorder; mania; depression; HLA cluster.

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List of Abbreviations

Abbreviation	Meaning
APA	American Psychiatric Association
BD	Bipolar Disorder
BP	Biological Processes
CADD	Combined Annotation Dependent Depletion
CC	Cellular Components
CTD	Comparative Toxicogenomic Database
DAVID	Database for Annotation, Visualization, and Integrated Discovery
DGIdb	Drug-Gene Interaction Database
DSigDB	Drug Signature Database
DSM	Diagnostic and Statistical Manual
FDR	False Discovery Rate
FUMA GWAS	Functional Mapping and Annotation of Genome-Wide Association Studies
G x E	Gene Environment Interaction
GENE2FUNC	Gene to Function
GO	Gene Ontology
GSEA	Gene Set Enrichment Analysis
GTEx	Genotype Tissue Expression
ICD	International Classification of Diseases
KEGG	Kyoto Encyclopedia of Genes and Genomes Pathway
MCC	Matthews Correlation Coefficient
MCODE	Molecular Complex Detection
MF	Molecular Function
MHC	Major Histocompatibility Complex

MTIs	MicroRNA-Target Interactions
PGC	Psychiatric Genomic Consortium
PPI	Protein-Protein Interaction
PRR	Pattern Recognition Receptors
SNP	Single-Nucleotide Polymorphism
SNP2GENE	SNP to Gene
STRING	Search Tool for Retrieval of Interacting Genes/Proteins
TF	Transcription Factor
TLR	Toll-Like Receptor

Flow Chart

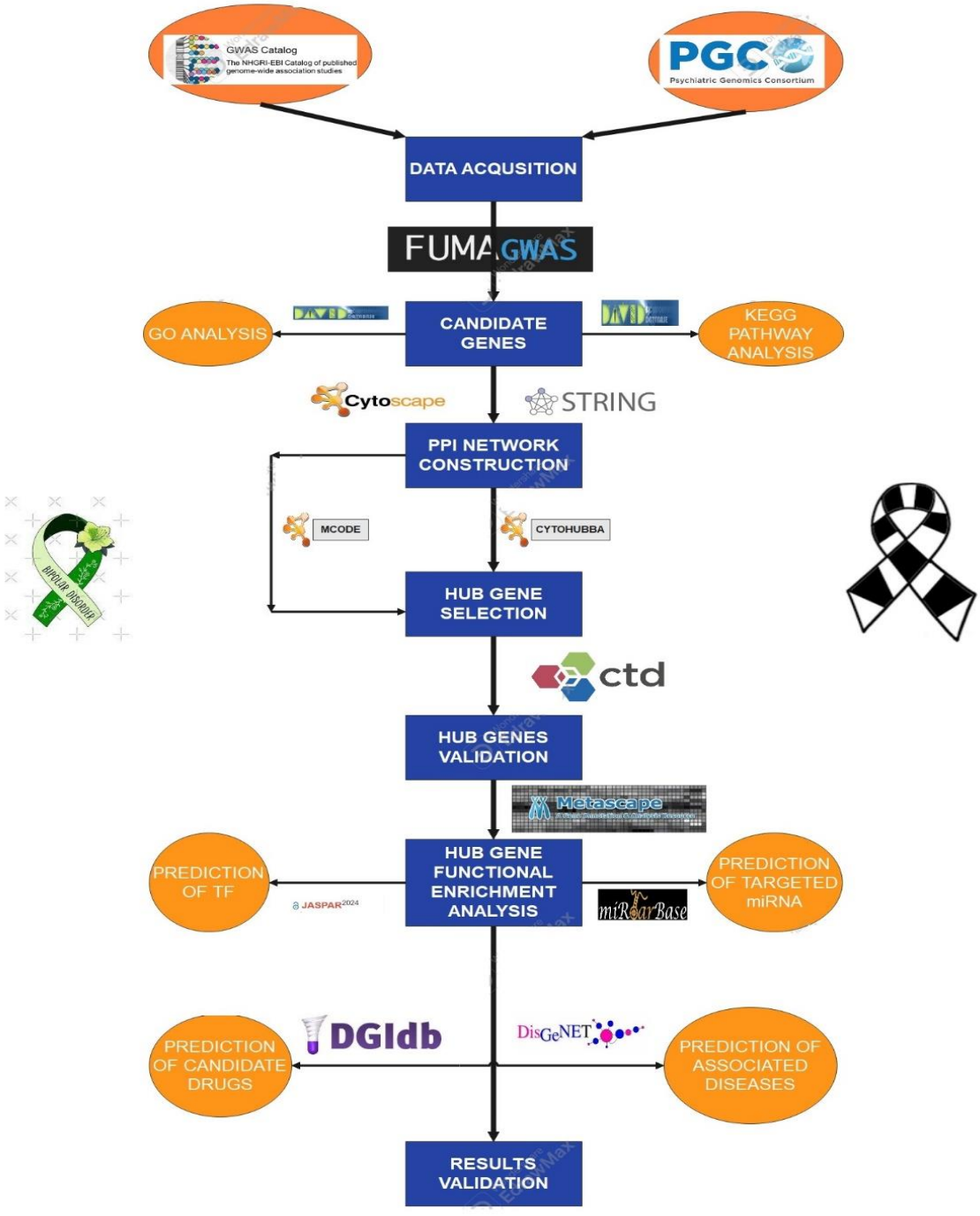


Fig. 1 | Research Flowchart

Introduction

Bipolar disorder **(BD)** is a mental health condition associated with severe shifts in mood. Even though it is often called a cycling illness, the periods of mania are actually what really define it. This is important because a doctor will usually diagnose someone as having bipolar disorder if they have gone through a stage of high energy and overly excited mood, even though they might not have had any depressions. Notably, patients manage to return to their usual selves during the breaks between these extreme mood stages.

During a manic episode, delusions and hallucinations may or may not occur. However, when experiencing a depressive episode, individuals may exhibit signs of depression such as a low mood, loss of interest, and reduced sexual desire. These can also be accompanied by a decrease in self-confidence, energy, and feelings of guilt and worthlessness. Both mania and depression can severely influence social and occupational functioning (Maj et al., 2002). It is important to note that bipolar disorder is associated with a high morbidity and mortality rate, with a study suggesting a 20 times greater risk for suicide compared to the general population (Perlis et al., 2010).

The early-onset of bipolar disorder can lead to long-lasting and severe clinical symptoms, an increased genetic susceptibility to mood disorders, and poor clinical outcome. Previous studies have indicated that the typical age of occurrence is between early-to-mid 20s to early 30s (Kessing et al., 2021).

Aim of Study:

The objective of this study is to utilize GWAS studies and conduct a comprehensive bioinformatics analysis in order to delve into the underlying molecular mechanisms of BD and uncover potential diagnostic markers. Additionally, this research will investigate potential therapeutic targets for BD.

Research Problem:

Bipolar Disorder is a chronic disease that requires a lifetime treatment. In this study, we will try to identify the potential genetic diagnostic markers in order to try to discover new potential treatments that do not only manage the disease symptoms, but also may find the underlying mechanisms for BD that may help reducing the lifetime treatment and even help curing the disease on genomic level utilizing advanced bioinformatics tools.

Research Hypothesis:

Establishing the association between HLA-gene cluster and histone- gene cluster and BD, which remains not fully understood. Besides, the potential treatment based on this association may open a new door for managing the disease in a way that reduces the lifetime treatment. In addition, the comorbidity of autoimmune diseases and other psychiatric disorders may help treatment development.

Chapter One: Theoretical Background:

➢ Symptoms:

✓ *Mania and Hypomania:*

The key defining characteristic of mania syndrome is a prolonged period of heightened mood, lasting for a minimum of seven days (or any length of time while hospitalized). Along with several other symptoms that can vary in severity: feelings of inflated self-worth, unrealistic optimism or grandiose beliefs, rapid and pressured speech, racing thoughts that are difficult to control, distractibility, surge of energy and restlessness, decreased interest in sexual activities, overactive or agitated behavior and impulsiveness without consideration of consequences. All of these symptoms cause an impairment in social or occupational functioning.

The states of hypomania and mania are both characterized by elevated energy and mood, yet they differ in their severity, duration, impact, and treatment. Hypomania, a less severe form of mania, typically lasts for a minimum of four consecutive days, does not significantly impact social or occupational functioning, and usually does not require hospitalization. On the other hand, mania, a more severe manifestation, typically lasts for at least one week, greatly impairs social and occupational functioning, and may require hospitalization (Goodwin et al., 2002).

However, the distinction between the two is not always clear and has been a subject of study among researchers.

✓ *Depression:*

While there are some similarities in clinical features between depressive episodes in bipolar disorder and unipolar depression, there are also important differences that can help distinguish between the two. One key difference is the presence of manic symptoms in bipolar depression; these episodes can sometimes even reach psychosis. In contrast, unipolar depression is characterized by depressive symptoms without these manic features. Another factor to consider is family history. Bipolar depression is more likely to be observed in individuals with a family history of bipolar disorders, whereas this is less common in unipolar depression. Additionally, individuals with bipolar depression tend to experience more lifetime affective episodes, including both depressive and manic or hypomanic episodes, compared to those with unipolar depression. It is also worth noting that age may play a role, as bipolar depression is often first

diagnosed in younger individuals while unipolar depression can occur at any age (Ghaemi et al., 2004).

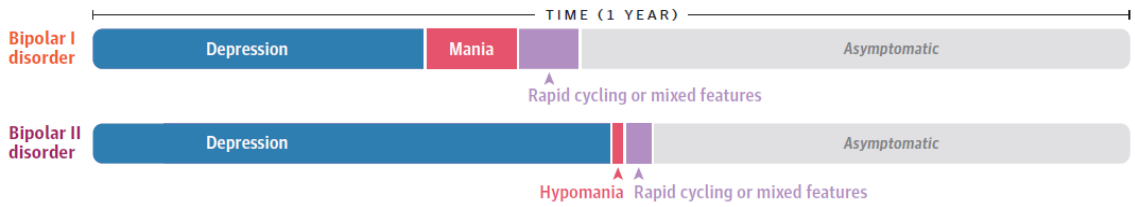


Fig. 2 | Percentage of Weeks Spent with Specific Mood Symptoms and Asymptomatic During Long-Term Follow-up of BD Subtypes. [17]

✓ *Mixed States:*

The complexity of bipolar disorder reaches its maximum in mixed states. These states encompass a broad range of behavioral and emotional disturbances, with pure depression and pure mania serving as the prototypical endpoints on a continuum. The idea of mixed states was first introduced by Kraepelin and Weygandt, who noted a blending of three dimensions: mood, thinking, and psychomotor activity. (Marneros et al., 2001).

In their observation, there are six distinct subtypes of mixed states: depression with flight of ideas, excited depression, depressive-anxious mania, mania with thought poverty, inhibited mania, and manic stupor.

In the DSM-IV, a patient is categorized as experiencing a mixed episode if they meet the criteria for both a manic episode and a major depressive episode for at least one week. This can manifest as rapid and alternating shifts between moods of sadness, irritability, and dysphoria. Slightly deviating from this definition, some refer to this as "mixed mania" when a manic episode also includes full syndromal depression, although this is not commonly seen in clinical settings. However, this definition may not account for individuals who have a combination of syndromal and sub-syndromal symptoms from either end of the mood spectrum. For example, "mixed mania" could also include experiencing isolated depressive symptoms during a manic episode, while "mixed depression" could involve having some manic symptoms while in a major depressive episode. Thus, the prevalence of these types of mixed episodes could be greater when considering a broader definition. (Swann et al., 2000)

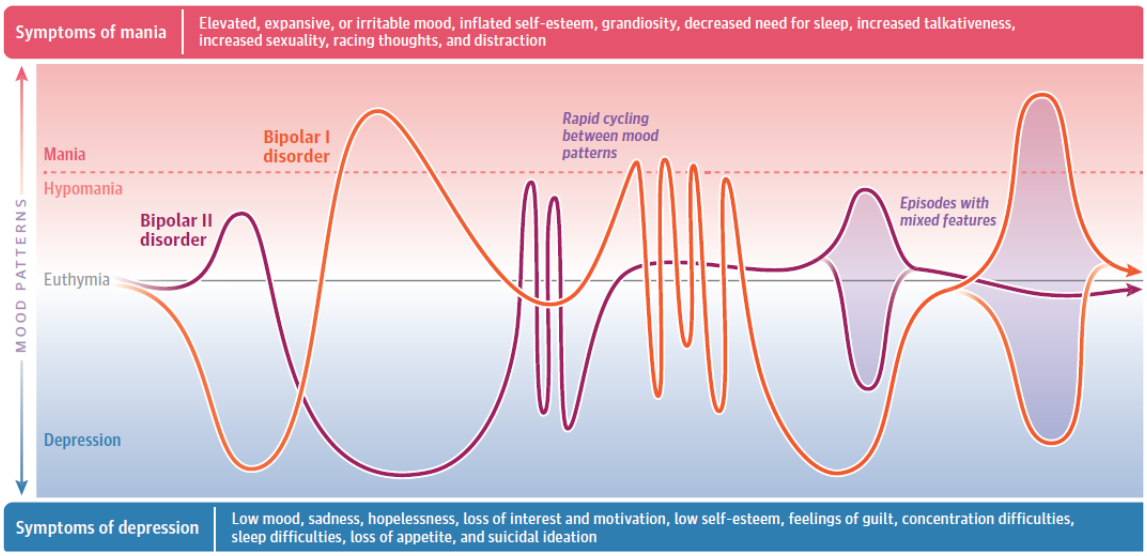


Fig. 3 | Sample Mood Patterns in BD Subtypes.[17]

➢ Epidemiology:

According to the DSM-IV criteria, the National Comorbidity Study replication found that both men and women have similar lifetime prevalence rates of BD-I (1.0%) and BD-II (1.1%). The study also showed that sub-threshold symptoms of hypomania, classified as bipolar spectrum disorder, are more commonly reported with a prevalence rate of 2.4%. When looking at incidence rates, which mainly focus on BD-I, it is estimated to be around 6.1 per 100,000 person years (with a 95% confidence interval of 4.7 to 8.1). However, the method of diagnosis (such as lay interviewers vs. clinically trained ones) and the racial, ethnic, and demographic context can have a moderate impact on the estimates of both incidence and lifetime prevalence of bipolar disorder. For example, countries with higher income and more westernized lifestyles tend to have slightly higher rates of bipolar disorder (estimated at 10% higher than other countries). (Goes et al., 2023)

➢ Comorbidity:

It is rare for bipolar disorder to occur on its own, as there is a high likelihood of co-occurring symptoms and disorders throughout a person's lifetime. This includes elevated risk for issues such as anxiety, attentional disorders, substance misuse, and personality disorders. The reasons for this comorbidity can be diverse and complex. These factors may stem from how diagnostic criteria currently classify disorders, the possibility of multiple

independent illnesses, or the ripple effects of one disorder increasing the chances of developing another. (Merikangas et al., 2011)

Research suggests that anxiety disorders often arise before the manifestation of clear manic or hypomanic symptoms, implying that they may be indicative of prodromal symptoms that arise earlier in life. This pattern is also seen in individuals with bipolar disorder and attention deficit/hyperactivity disorder, where subthreshold and syndromic symptoms are observed throughout the lifespan, but are particularly prevalent in those with early onset bipolar disorder. In contrast, alcohol and substance misuse disorders are present both before and after the onset of bipolar disorder, indicating a more reciprocal relationship between the two conditions. (Sandstrom et al., 2021)

On the other hand, similar to other serious mental disorders, bipolar disorder is known to be linked to a higher incidence of common medical conditions like obesity, high cholesterol, heart disease, lung disease, and thyroid problems. This is likely due to factors such as a sedentary lifestyle, unhealthy eating habits, smoking, and substance abuse. However, certain medications used to treat bipolar disorder may also play a role in these health issues. Due to this added medical burden and limited access to proper care, people with bipolar disorder have a mortality rate that is about 2.6 times higher than the general population. Therefore, it is crucial to prioritize the use of treatments with more favorable long-term side effect profiles to address this issue. (Launders et al., 2022)

- **Genetic Insight:**

Since its inception, it has been observed that bipolar disorder frequently runs within families. In fact, family history is the most significant risk factor for the development of this disorder. First degree relatives have an eightfold increased risk compared to the baseline population rate of 1%. (Smoller & Finn et al., 2003)

While genetic, behavioral, and cultural transmission cannot be fully distinguished in family studies, twin and adoption studies have confirmed that genetics play a major role, with estimates of heritability ranging from 60% to 80%. Studies on BD-II have been limited, but its heritability has been found to be around 46%, closer to that of more common disorders like major depressive disorder or general anxiety. (Mullins et al., 2021)

However, simply having a high level of heritability does not necessarily mean that there are genes with major impacts on bipolar disorder. New research suggests that the genetic risk for this disorder is more likely spread out among numerous common variants with smaller effects. Further investigation into rare variations has indicated some slightly more influential variants, which seem to align with common variations in genes related to the synapse and the postsynaptic density. Although it is unlikely that testing individual variants or genes will be effective for diagnostic purposes, there is potential in utilizing polygenic risk studies. These studies have the ability to combine risk loci and differentiate between cases and controls, although primarily at the group level rather than the individual level. Furthermore, these risk scores can be applied to not only identify shared genetic risk factors among medical and psychiatric disorders, but also to determine coinheritance patterns. In particular, bipolar disorder (BD-I) has a strong genetic correlation with schizophrenia and major depressive disorder, with a genetic correlation of 0.69 and 0.48, respectively. It is worth noting that BD-I exhibits a stronger correlation with schizophrenia while BD-II is more closely correlated with major depressive disorder, with a correlation of 0.66. Additionally, a lower level of coinheritance was observed in other disorders. (Murray et al., 2021)

These correlations offer proof that there are common genetic risk factors for both bipolar disorder and other major psychiatric disorders. This conclusion is further supported by recent family studies conducted through nationwide registries. However, while polygenic risk scores have the potential to be beneficial, their interpretation must be approached with care due to their limited representation in the general population and lingering concerns around potential confounding factors such as gene-environment correlations.

- **Environmental Risk Factors:**

Due to the challenging nature of quantifying and investigating the significant and frequently shared environmental risk elements of a complex disorder such as bipolar disorder, there has been relatively limited exploration into the role of these risk factors in the development or

alteration of the disorder. While evidence for prenatal risk factors is varied, it is not as convincing as the evidence found in other disorders such as schizophrenia. Additionally, initial findings suggest a potential correlation between significant seasonal fluctuations in solar radiation and an earlier manifestation of bipolar disorder, potentially due to its impact on circadian rhythm. This link may also increase the likelihood of experiencing a depressive episode during the onset of the disorder (Bauer et al., 2022)

The central area of focus within environmental studies has been on the impact of traumatic and stressful experiences during early childhood and adulthood. These adverse events have been linked to a variety of complex effects, including a younger age of onset for bipolar disorder, more severe illness progression, increased occurrence of psychotic symptoms, substance abuse, comorbid psychiatric disorders, and a heightened risk of suicide attempts. Surprisingly, evidence shows that positive life events, specifically those related to achieving personal goals, can also contribute to the development of elevated states in bipolar disorder. (Agnew-Blais & Danese et al., 2016)

- **Diagnosis:**

  - ✓ *Bipolar I Disorder:*

To receive a bipolar I disorder diagnosis, one must fulfill the criteria for a manic episode as outlined in the DSM-V. This manic episode may have been preceded or followed by episodes of hypomania or major depression.

A manic episode is characterized by an extended period of elevated or irritable mood, as well as heightened levels of activity or motivation lasting at least one week. This state of mood disturbance and increased energy must be present for most of the day, nearly every day, or for any length of time if hospitalization is necessary. During this time, a person may experience at least 3 (or 4 if irritable mood is present) symptoms to a significant degree that are noticeably different from their usual behavior. Some of these symptoms include an inflated sense of self-importance, a decreased need for sleep, excessive talking or feeling pressure to continue talking, racing thoughts, or difficulty focusing. Engaging in risky behaviors with the potential for negative consequences, such as impulsive spending, sexual recklessness, or unwise business decisions is a sign of excessive involvement. This level of involvement can have a significant impact on

one's ability to function in social or work settings, and may even require hospitalization to prevent harm to oneself or others. Additionally, it is important to note that these behaviors are not a result of substance use or another medical condition.

- ✓ *Bipolar II Disorder:*

The individual meets the criteria for at least one episode of hypomania and at least one episode of major depression. A manic episode has never been observed. The occurrence of these episodes cannot be attributed to schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder, or other unspecified schizophrenia spectrum and other psychotic disorders. The symptoms of depression or the volatility resulting from frequent shifts between depression and hypomania significantly disrupt the individual's social, occupational, or other important areas of functioning.

➢ Treatment:

- ✓ *Pharmacological Therapy:*

Pharmacological therapy must be personalized based on the individual's specific symptoms (depression or hypomania/mania). The primary objective is to decrease the intensity of the current mood episode and prevent future episodes from occurring with the least severity. To achieve this goal, clinical guidelines suggest the use of mood stabilizers like lithium, valproate, and lamotrigine, as well as atypical antipsychotic medications like quetiapine, aripiprazole, and cariprazine, for both short-term and long-term management of bipolar disorder. (Nierenberg et al., 2023)

1. Lithium:
   Considered the most established mood stabilizer for BD, lithium has consistently proven its effectiveness in treating acute episodes of both depressive and manic episodes. As a preventive measure, research has also shown its efficacy in reducing the risk of relapse for both types of episodes. Furthermore, this powerful medication has been linked to a decreased likelihood of suicidal thoughts among BD patients.
2. Divalproex:
   Along with its formulations of sodium valproate and valproic acid, Divalproex has proven to be a valuable tool in managing BD. Extensive research has shown its effectiveness in treating acute

mania and mixed episodes, but there is less substantial evidence for its use in acute depression compared to lithium. Additionally, it has demonstrated efficacy in preventing both mania and depression when utilized in the maintenance phase.

3. Lamotrigine:
   Studies have demonstrated the effectiveness of lamotrigine in treating bipolar depression and preventing relapse of depression. Nevertheless, one must be cautious of its potential adverse reactions, such as skin rash, including the severe conditions of Stevens-Johnson syndrome and toxic epidermal necrolysis.
4. Carbamazepine:
   The effectiveness of carbamazepine in treating acute bipolar mania and preventing future relapses has been proven. However, before prescribing it, the clinician must carefully consider the patient's medical history, paying particular attention to any previous blood disorders or liver problems.
5. Antipsychotics:
   In the last decade, there has been a surge of large-scale, well-designed studies exploring the effects of several atypical antipsychotics, including olanzapine, quetiapine, aripiprazole, risperidone, paliperidone, amisulpiride, asenapine, ziprasidone, and haloperidol, on managing bipolar depression, mania, and maintenance treatment. The findings from these studies are indicative of the effectiveness of olanzapine, quetiapine, aripiprazole, risperidone, paliperidone, and ziprasidone in addressing acute manic episodes. In fact, evidence exists to support the use of quetiapine as a standalone treatment, as well as the combination of olanzapine and fluoxetine in tackling bipolar depression. Furthermore, emerging data also points to the potential effectiveness of lurasidone in managing acute episodes
6. Antidepressants:
   For years, antidepressants have been the conventional treatment for bipolar depression. Nevertheless, the past 20 years have seen considerable debate arise around their usage due to the potential risk of triggering manic or hypomanic episodes. Recent metanalysis findings point to a preferable outcome with the incorporation of antidepressants into a treatment plan that also includes mood stabilizers. This approach not only surpasses the efficacy of using a

mood stabilizer alone, but it also has no discernible impact on the likelihood of a manic switch.

7. Benzodiazepines:

   Recent studies have assessed the effectiveness of including benzodiazepines such as clonazepam and lorazepam alongside lithium. The current evidence indicates that it can be challenging to distinguish the antimanic effects of these agents from their sedative effects. Therefore, they are typically seen as supplementary treatments, which may be helpful in treating acute episodes. Additionally, there is evidence supporting the potential benefits of using lorazepam to address agitation. (Shah et al., 2017)

✓ *Psychotherapy:*

Optimal management of bipolar disorder involves a combination of psychopharmacological and psychosocial treatment, as stated by guidelines. A key aspect of this treatment is psychoeducation, which involves providing individuals with crucial information about the disorder, the importance of adhering to medication, recognizing early signs of mood episodes, and developing strategies for managing symptoms. Additionally, educating individuals about potential adverse effects of medication has been shown through numerous studies to result in lower relapse rates, longer periods of remission, decreased severity of manic and depressive symptoms, fewer hospitalizations, and better treatment adherence in comparison to nonstructured interventions that do not include psychoeducation. (Nierenberg et al., 2023)

Chapter Two: Materials and Methods:

1. Data Acquisition:

The data for this study was gathered from two distinct sources: the **P**sychiatric **G**enomic **C**onsortium **(PGC)** and the **GWAS** Catalog. The PGC is a reputable organization (https://pgc.unc.edu/) that conducted the third GWAS meta-analysis of their Bipolar Disorder Working Group. This meta-analysis included data from various European, North American, and Australian BD cohorts. The PGC BD dataset consisted of 57 cohort studies, with a total of 41,917 individuals diagnosed with BD (cases) and 371,549 controls. Similarly, the PGC BD Ⅰ dataset included data from 55 cohorts, with a total of 25,060 individuals diagnosed with BD Ⅰ (cases) and 449,978 controls. Lastly, the PGC BD Ⅱ dataset included data from 31 BD Ⅱ cohorts, with a total of 6,781 individuals diagnosed with BD Ⅱ (cases) and 364,075 controls.

As for **GWAS** Catalog, on November 4th 2023, a search was conducted using "bipolar disorder" as the query term. This yielded a total of 126 studies, from which 69 studies were selected for further examination. In total, these 69 studies revealed 682 associations linked to 806 potential SNPs.

The overall final number of candidate SNPs equals 3,146,315 was utilized as the input data for the subsequent step.

2. SNPs Annotation:

With the aim of prioritizing genes based on GWAS findings, the **FUMA-GWAS** platform (**F**unctional **M**apping and **A**nnotation of **G**enome-**W**ide **A**ssociation **S**tudies) (http://fuma.ctglab.nl) was employed. This resource greatly assists the interpretation and visualization of GWAS results by incorporating functional and biological information (Watanabe et al., 2017). The first step in using FUMA involved the input of candidate SNPs, along with their chromosomal location and P-value, into the SNP2GENE function. The parameters were set as follows: a sample size of 3,146,315, a P-value threshold less than (5e-8) for genome-wide significance, a CADD score of more or equal (12.37) for both positional and eQTL mapping, and protein coding regions were used as the default gene types. The MHC region was not excluded and a MAGMA Analysis was also performed.

The second step was performing GENE2FUNC function taking the list of genes IDs as identified by SNP2GENE to annotate genes in biological context.

3. Functional Enrichment of Gene Sets:

DAVID the **D**atabase for **A**nnotation, **V**isualization and **I**ntegrated **D**iscovery (https://david.ncifcrf.gov/) is a web server that offers a comprehensive platform for functional enrichment and annotation of gene lists (Sherman et al., 2022).. Through its user-friendly interface, DAVID allows for in-depth exploration of gene ontology (GO), covering three fundamental aspects: biological processes (BP), cellular components (CC), and molecular functions (MF). Moreover, the server also employs the Kyoto Encyclopedia of Genes and Genomes (KEGG) to provide insight into complex signaling pathways, enhancing our understanding of gene function.

4. PPI Network Construction & Analysis:

The **STRING** Search Tool, accessible at (https://www.stringdb.org) **S**earch **T**ool for **R**etrieval of **I**nteracting **G**enes/Proteins, is a valuable resource for identifying interacting genes and proteins. It combines both known and predicted protein-protein interactions, encompassing both direct physical connections and indirect functional associations (Szklarczyk et al., 2016). By leveraging the genes identified through Fuma analysis, we constructed a comprehensive protein-protein interaction (PPI) network. This network was tailored with the parameters set to the following key features: Organism: Homo sapiens, Network type: full STRING network, Required score: highest confidence (0.900), FDR stringency: medium (5 percent).

5. PPI Network Module Analysis & Hub Genes Selection:

The generated network was exported to **Cytoscape Software** (version 3.10.1) (http://cytoscape.org), an open-source project that integrates biomolecular interaction networks (Shannon et al., 2003). The **CytoHubba** plug-in was then utilized to calculate connectivity scores and determine the intersections among the top thirty genes.

To identify the central hub genes, a four-fold algorithm was employed, utilizing two local-based methods (MCC and Degree) and two global-based methods (Stress and Radiality). A Venn diagram was also created using Venny (2.1) (https://bioinfogp.cnb.csic.es/tools/venny/) to visualize any overlap among the four algorithms. Furthermore, to uncover connected areas, we employed the **MCODE M**olecular **C**omplex **D**etection plug-in

within Cytoscape, effectively identifying possible protein clusters within the network. (Cao et al., 2018)

6. Validation of Hub Genes:

The use of **C**omparative **T**oxicogenomic **D**atabase **(CTD)** (https://ctdbase.org/) was essential in verifying the connection between the hub genes and **BD**. This highly reliable database provides comprehensive, manually curated data on chemical-gene/protein interactions, chemical-disease relationships, and gene-disease relationships, ensuring the robustness of our findings (Davis et al., 2023).

7. Hub Genes Functional Enrichment Analysis:

Metascape (http://metascape.org/), a gene annotation and analysis resource, is a biologist-oriented resource that provides a comprehensive analysis of gene lists. It combines functional enrichment, interactome analysis, gene annotation, and membership search to cover over 40 independent knowledgebases within one integrated portal. It facilitates comparative analyses of datasets across multiple independent and orthogonal experiments. (Zhou et al., 2019).

In order to gain new insights, it was utilized to analyze functional enrichment of the hub genes.

8. Prediction of Target miRNAs:

With over 360,000 experimentally validated microRNA-target interactions (MTIs) collected from diverse sources, **miRTarBase** (https://mirtarbase.cuhk.edu.cn/) stands as a premiere, curated database for exploring various miRNA-disease, miRNA-site, and microRNA-expression associations. Along with powerful features such as a word cloud showcasing miRNA-disease information and a CLIP-seq data viewer for miRNA-target sites, users can also access clinical microRNA and gene expression profiles from TCGA. (Chou et al., 2015b)

It was utilized to predict target miRNA. Furthermore, to determine the differences in miRNA expression between BD patients and healthy individuals, a thorough investigation was carried out. This involved extracting a list of miRNAs that had been previously confirmed in six studies, as documented in Pubmed (Ceylan et al., 2020; Lee et al., 2020; Y. Chen et al., 2020; Fu et al., 2021; Camkurt et al., 2020; Fries et al., 2018). The goal of this process was to examine how these specific miRNAs were expressed in the context of BD.

9. Prediction of Target Transcription Factors (TFs):

JASPAR (https://jaspar.elixir.no/) is one of the largest open-access databases of curated transcription factor (TF) binding profiles for TFs from six different taxonomic groups (Castro-Mondragón et al., 2021). It is integrated in **miRNet** and was employed to predict the target transcription factors associated with hub genes.

10. Analysis of Gene-Disease Association:

NetworkAnalyst (3.0) (https://www.networkanalyst.ca/) is a revolutionary online visual analytics platform designed for analyzing transcriptome data, conducting network analysis, and performing meta-analysis on gene expression. Furthermore, it can be used to explore the biological significance of gene lists by investigating protein-protein interaction networks, pathways and ontologies, along with performing statistical tests, visualizations, and network-based approaches. (Zhou et al., 2019).

DisGeNET DB **D**isease **G**ene Network **D**atabase (http://www.disgenet.org/) is a large-scale discovery platform that integrates human gene and variant-disease associations from various expert curated databases and scientific literature, it includes Mendelian, rare, complex and environmental diseases, as well as abnormal phenotypes and traits. (Chen et al., 2023).

DisGeNET server through NetworkAnalyst was utilized to investigate the gene-disease association to find molecular basis of **BD** and its comorbidities.

11. Analysis of Candidate Drugs:

The **D**rug-**G**ene **I**nteraction **D**atabase (**DGIdb**), found at(www.dgidb.org), offers valuable insight into the relationships between drugs and genes. By compiling data from various publications, databases, and web sources, it provides users with a comprehensive understanding of druggable genes and their potential interactions. The database organizes drug, gene, and interaction data into easily digestible concepts, making it easily accessible through a user-friendly search interface, API, and TSV data downloads. (Freshour et al., 2020)

Coremine Medical (www.coremine.com) is a pioneering domain-specific information community powered by the COREMINE Platform, specifically crafted for medical information, with a one-of-a-kind search approach that provides users with specialized networks. These networks highlight the

links between a search query and related topics like diseases, medications, alternative remedies, treatments, and potential side effects. The data comes from reputable medical databases, empowering users to explore the networks and access relevant documents from diverse sources.

It was utilized to explore networks involving gene, proteins, associated diseases and treatments. (Ignatieva et al., 2017)

Chapter Three: Results & Discussion:

1. SNPs Annotation:

By incorporating SNP2FUNC, the input SNPs were successfully mapped to a total of 5506 protein coding genes. Notably, the significance threshold for the entire genome was illustrated by the red dashed line in **(Figure_4)**, precisely defined as a P-value of (9.081e-6).
The findings of our study revealed the following summary results: a total of 118 genomic risk loci, 138 lead SNPs, 190 independent significant SNPs, 13008 candidate SNPs, and 539 mapped genes. These results are presented in detail in **(Table_1).**

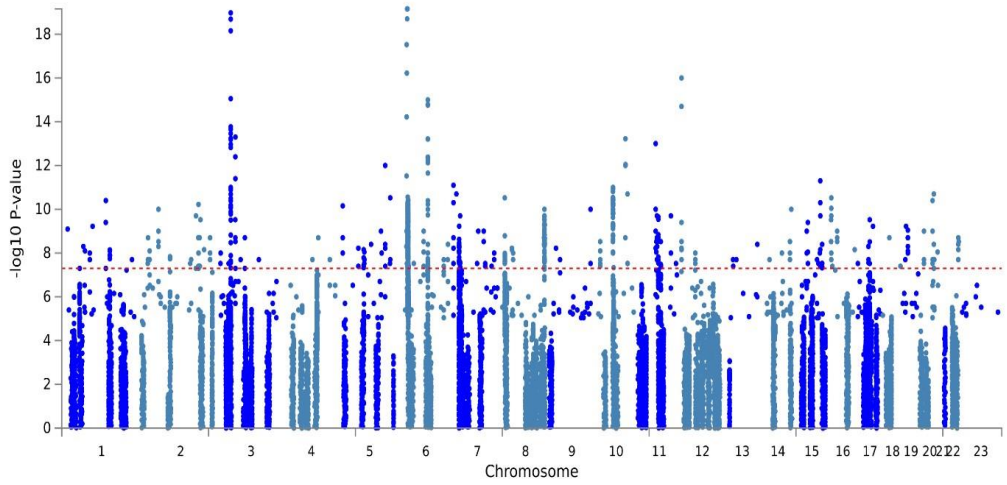


Fig. 4 | Manhattan Plot of Genome-Wide Association Meta-Analysis of 41,917 BD Cases and 371,549 Controls. The x Axis Shows Genomic Position (chromosomes 1–22 and X), and the y Axis Shows Statistical Significance as –log10 [*P* value] via Fuma GWAS

Table 1 | Genome-Wide Significant Loci for BD from Meta-Analysis

No	Genomic Locus	uniqID	rsID	chr	pos	p	nIndSig SNPs	IndSigSNPs
1	1	1:19992066:C:T	rs10917509	1	19992066	8.00E-10	1	rs10917509
2	2	1:73725998:G:T	rs12136984	1	73725998	5.00E-09	1	rs12136984
3	3	1:79238015:C:T	rs4650608	1	79238015	8.00E-09	1	rs4650608
4	4	1:95578207:C:T	rs12563424	1	95578207	1.00E-08	1	rs12563424
5	5	1:105153596:C:T	rs140700006	1	1.05E+08	6.00E-10	1	rs140700006
6	6	1:150143302:A:G	rs78676616	1	1.5E+08	4.00E-11	1	rs78676616
7	7	1:163745389:C:T	rs10737496	1	1.64E+08	7.17E-09	1	rs10737496
8	8	1:239210058:A:C	rs72769124	1	2.39E+08	2.00E-08	1	rs72769124
9	9	2:21531266:A:G	rs1510606	2	21531266	3.00E-08	1	rs1510606
10	10	2:22604140:A:G	rs2339519	2	22604140	2.00E-09	2	rs2339519; rs11887562
11	11	2:28033538:A:C	rs12474906	2	28033538	8.00E-09	1	rs12474906
12	11	2:28113911:A:G	rs2305929	2	28113911	2.00E-08	1	rs2305929
13	12	2:57987593:C:T	rs11682175	2	57987593	1.00E-09	1	rs11682175
14	12	2:58071593:A:C	rs80256351	2	58071593	1.00E-10	1	rs80256351
15	13	2:97392778:A:G	rs72809828	2	97392778	1.46E-08	1	rs72809828
16	14	2:166152389:A:G	rs17183814	2	1.66E+08	3.00E-08	1	rs17183814
17	15	2:169481837:C:G	rs13417268	2	1.69E+08	2.00E-08	1	rs13417268
18	16	2:185811940:A:C	rs4380187	2	1.86E+08	2.00E-10	1	rs4380187
19	17	2:193848340:A:C	rs59979824	2	1.94E+08	2.00E-09	2	rs59979824; rs2011302
20	18	2:194378649:A:C	rs2439202	2	1.94E+08	6.00E-11	1	rs2439202
21	19	2:198304577:A:G	rs6434928	2	1.98E+08	2.00E-09	2	rs6434928; rs34388051
22	20	2:201160771:C:T	rs1367858	2	2.01E+08	3.00E-10	1	rs1367858
23	21	2:213504684:C:T	rs7587236	2	2.14E+08	7.00E-09	1	rs7587236
24	22	2:233790475:A:G	rs2880986	2	2.34E+08	2.00E-09	1	rs2880986
25	23	3:1897973:C:T	rs2727943	3	1897973	3.00E-08	1	rs2727943
26	24	3:2538446:A:G	rs34771152	3	2538446	1.00E-08	1	rs34771152
27	25	3:29749358:A:G	rs1440518	3	29749358	3.00E-08	1	rs1440518
28	26	3:36870230:A:G	rs11129735	3	36870230	1.06E-19	5	rs11129735; rs17035750; rs13314421; rs55644704; rs4624519

29	27	3:52837793:C:T	rs4481150	3	52837793	5.00E-14	2	rs4481150;rs7618915
30	28	3:70488788:A:T	rs115694474	3	70488788	2.00E-08	1	rs115694474
31	29	3:85052150:C:T	rs9831123	3	85052150	2.00E-09	1	rs9831123
32	30	3:132612664:C:T	rs9863544	3	1.33E+08	2.00E-08	1	rs9863544
33	31	4:103198082:A:G	rs13135092	4	1.03E+08	2.00E-08	1	rs13135092
34	32	4:123076007:A:G	rs112481526	4	1.23E+08	2.00E-09	1	rs112481526
35	33	4:162294038:C:T	rs11724116	4	1.62E+08	2.00E-08	1	rs11724116
36	34	5:7533985:C:T	rs78308718	5	7533985	7.00E-11	1	rs78308718
37	35	5:60643513:C:T	rs6449529	5	60643513	6.00E-09	1	rs6449529
38	36	5:78848357:A:G	rs11744542	5	78848357	7.00E-09	1	rs11744542
39	37	5:80961069:A:G	rs6887473	5	80961069	9.00E-09	2	rs6887473; rs10035291
40	38	5:104037760:A:G	rs12055234	5	1.04E+08	4.00E-09	1	rs12055234
41	39	5:137712121:C:T	rs10043984	5	1.38E+08	1.00E-09	1	rs10043984
42	40	5:140107231:C:T	rs6875495	5	1.4E+08	1.00E-08	1	rs6875495
43	41	5:152187123:G:T	rs72799190	5	1.52E+08	1.00E-12	1	rs72799190
44	41	5:152540354:C:T	rs2910032	5	1.53E+08	4.00E-08	1	rs2910032
45	42	5:168466569:G:T	rs7720655	5	1.68E+08	3.00E-08	1	rs7720655
46	43	5:169289206:C:T	rs10866641	5	1.69E+08	3.00E-11	2	rs10866641; rs11742527
47	44	6:25384361:C:T	rs215011	6	25384361	5.00E-09	1	rs215011
48	44	6:26364056:C:T	rs10946817	6	26364056	1.00E-09	1	rs10946817
49	44	6:26408551:G:T	rs75782365	6	26408551	3.00E-18	1	rs75782365
50	44	6:27311658:A:G	rs6922815	6	27311658	3.00E-08	1	rs6922815
51	44	6:27805255:A:C	rs34706883	6	27805255	7.00E-20	1	rs34706883
52	44	6:29244219:G:T	rs144447022	6	29244219	7.00E-20	5	rs144447022; rs2517664; rs1541269; rs2523735; rs2517613
53	44	6:30154199:C:T	rs2074473	6	30154199	1.60E-09	4	rs2074473; rs2517613; rs1264372 rs2523735
54	44	6:30796545:C:T	rs1264350	6	30796545	5.76E-11	15	rs1264350; rs3130781; rs3131934; rs1628680; rs3130985;

								rs3131006; rs2523599; rs2853949; rs1811197; rs2517664; rs1541269; rs2523735; rs2517613; rs3094035; rs1264372
55	44	6:30932309:C:T	rs2844697	6	30932309	1.93E-08	8	rs2844697; rs1628680; rs3130985; rs2853949; rs1811197; rs3094035; rs1264372; rs3130781
56	44	6:31881309:C:T	rs532086	6	31881309	3.83E-11	9	rs532086; rs3130781; rs3131934; rs1628680; rs3130985; rs3131006; rs2523599; rs2853949; rs1811197
57	44	6:32212264:A:G	rs427037	6	32212264	3.54E-10	3	rs427037; rs9357138; rs41315395
58	45	6:33537546:A:G	rs169738	6	33537546	4.00E-08	1	rs169738
59	46	6:50816718:A:G	rs55648125	6	50816718	3.00E-08	1	rs55648125
60	47	6:83953276:C:T	rs1180221	6	83953276	3.00E-09	1	rs1180221
61	47	6:84373058:A:G	rs60730	6	84373058	1.00E-09	1	rs60730
62	48	6:98565211:C:T	rs1487445	6	98565211	1.00E-15	5	rs1487445; rs6931604; rs17814604;rs17813294; rs62422661
63	49	6:152793572:A:T	rs4331993	6	1.53E+08	2.00E-08	1	rs4331993
64	50	6:166155457:A:G	rs1039002	6	1.66E+08	2.00E-08	1	rs1039002

65	51	6:166995260:C:G	rs10455979	6	1.67E+08	4.00E-09	1	rs10455979
66	52	7:1896413:A:G	rs4236274	7	1896413	8.00E-12	1	rs4236274
67	53	7:11871787:A:G	rs113779084	7	11871787	2.00E-11	1	rs113779084
68	54	7:21492589:A:G	rs6954854	7	21492589	5.94E-10	1	rs6954854
69	55	7:24775514:A:G	rs116052126	7	24775514	2.00E-10	1	rs116052126
70	56	7:82422405:C:T	rs17156675	7	82422405	3.00E-08	1	rs17156675
71	57	7:86427626:A:G	rs12704290	7	86427626	1.00E-09	1	rs12704290
72	58	7:105048158:C:T	rs73188321	7	1.05E+08	1.00E-09	1	rs73188321
73	59	7:110189944:A:G	rs2966424	7	1.1E+08	3.00E-08	1	rs2966424
74	60	7:131870597:A:C	rs6946056	7	1.32E+08	4.00E-08	1	rs6946056
75	61	7:140666965:C:T	rs13236223	7	1.41E+08	1.00E-08	1	rs13236223
76	62	8:9763581:C:G	rs62489493	8	9763581	3.00E-11	5	rs62489493; rs7013693; rs4840464; rs28630503; rs3088186
77	63	8:34152492:A:G	rs2953928	8	34152492	6.00E-09	1	rs2953928
78	64	8:38284581:C:T	rs6984358	8	38284581	1.00E-08	1	rs6984358
79	65	8:143322470:C:T	rs4284148	8	1.43E+08	2.00E-09	1	rs4284148
80	66	8:145000321:C:T	rs6993953	8	1.45E+08	1.00E-10	2	rs6993953; rs61156785
81	67	9:23347865:C:G	rs12553324	9	23347865	6.00E-09	1	rs12553324
82	68	9:37090538:C:T	rs10973201	9	37090538	2.00E-08	1	rs10973201
83	69	9:141068624:C:T	rs11137399	9	1.41E+08	1.00E-10	1	rs11137399
84	70	10:18725985:C:T	rs7095057	10	18725985	3.00E-09	1	rs7095057
85	71	10:62322034:C:T	rs10994415	10	62322034	1.00E-11	2	rs10994415; rs10994318
86	72	10:64525135:C:T	rs10761661	10	64525135	4.65E-08	1	rs10761661
87	73	10:104621068:A:G	rs12241517	10	1.05E+08	6.00E-14	3	rs12241517; rs11191582; rs11191356
88	74	10:111648659:C:T	rs2273738	10	1.12E+08	2.00E-11	2	rs2273738; rs72830427
89	75	11:61618608:A:G	rs174592	11	61618608	1.00E-13	1	rs174592
90	76	11:63689879:C:T	rs7121067	11	63689879	9.00E-09	2	rs7121067; rs484201
91	76	11:64009879:A:G	rs4672	11	64009879	3.00E-09	1	rs4672
92	77	11:65854561:A:G	rs489337	11	65854561	2.00E-10	1	rs489337
93	77	11:66324583:C:T	rs678397	11	66324583	5.00E-09	2	rs678397; rs7122539

94	78	11:70559893:A:G	rs11601580	11	70559893	2.22E-10	2	rs11601580; rs72948949
95	79	11:79077193:A:G	rs12576775	11	79077193	3.00E-09	2	rs12576775; rs12289486
96	80	11:113392994:C:T	rs2514218	11	1.13E+08	2.00E-10	1	rs2514218
97	81	11:130811356:C:T	rs35774874	11	1.31E+08	3.00E-08	1	rs35774874
98	82	12:2408194:A:G	rs4298967	12	2408194	1.00E-16	3	rs4298967; rs4765913; rs2238044
99	82	12:2499849:A:C	rs740417	12	2499849	7.00E-09	1	rs740417
100	83	12:49389320:A:C	rs1054442	12	49389320	1.00E-08	1	rs1054442
101	84	13:31318308:A:C	rs3803277	13	31318308	2.00E-08	1	rs3803277
102	85	13:31843598:A:G	rs1924817	13	31843598	4.00E-08	1	rs1924817
103	86	13:42653437:A:C	rs1012053	13	42653437	2.00E-08	1	rs1012053
104	87	13:113869045:A:G	rs35306827	13	1.14E+08	4.00E-09	1	rs35306827
105	88	14:30187405:C:T	rs10149407	14	30187405	2.00E-08	1	rs10149407
106	89	14:72442612:A:G	rs7161596	14	72442612	1.00E-08	1	rs7161596
107	90	14:99712945:A:G	rs11624408	14	99712945	3.00E-09	1	rs11624408
108	91	14:104261723:A:C	rs722637	14	1.04E+08	1.00E-10	1	rs722637
109	92	15:38969545:C:T	rs6495988	15	38969545	1.00E-09	1	rs6495988
110	93	15:42902246:A:G	rs1197546	15	42902246	4.00E-10	2	rs1197546; rs112968809
111	94	15:74148432:C:T	rs28379895	15	74148432	2.00E-08	1	rs28379895
112	95	15:78908565:C:T	rs28681284	15	78908565	3.00E-08	1	rs28681284
113	96	15:83531774:A:T	rs62011709	15	83531774	1.00E-08	1	rs62011709
114	97	15:85109237:A:G	rs12906474	15	85109237	5.00E-12	2	rs12906474; rs61074241
115	98	15:91426560:A:G	rs4702	15	91426560	4.00E-09	1	rs4702
116	99	16:9230816:A:G	rs28455634	16	9230816	3.00E-10	1	rs28455634
117	100	16:9939960:A:C	rs9926049	16	9939960	3.00E-11	3	rs9926049; rs11648559; rs11647445
118	101	16:13749265:A:C	rs7499750	16	13749265	4.00E-08	1	rs7499750
119	102	16:29939877:A:G	rs12691307	16	29939877	1.00E-09	1	rs12691307
120	103	16:89632725:G:T	rs12932628	16	89632725	7.00E-09	1	rs12932628
121	104	17:1835482:C:T	rs4790841	17	1835482	3.00E-08	1	rs4790841
122	105	17:37846512:A:T	rs2517959	17	37846512	5.00E-09	1	rs2517959
123	105	17:38129841:A:T	rs11870683	17	38129841	2.79E-08	1	rs11870683
124	105	17:38220432:G:T	rs61554907	17	38220432	2.00E-08	1	rs61554907
125	106	17:42191893:G:T	rs228768	17	42191893	3.00E-10	2	rs228768;

								rs4473241
126	107	17:53367300:A:G	rs884303	17	53367300	6.00E-10	1	rs884303
127	108	18:60243876:A:G	rs11557713	18	60243876	4.00E-08	1	rs11557713
128	109	19:10770305:C:T	rs7248205	19	10770305	2.00E-08	1	rs7248205
129	110	19:13153035:A:G	rs4926298	19	13153035	6.00E-10	1	rs4926298
130	111	19:19358207:C:T	rs111444407	19	19358207	9.00E-10	1	rs111444407
131	112	20:14501141:A:T	rs6079469	20	14501141	1.00E-09	1	rs6079469
132	113	20:43682551:G:T	rs67712855	20	43682551	4.00E-11	2	rs67712855; rs6130764
133	113	20:43944323:A:G	rs6032110	20	43944323	2.00E-08	2	rs6032110; rs6130764
134	114	20:48049506:C:T	rs237475	20	48049506	2.00E-11	2	rs237475; rs6125656
135	115	20:49610597:C:T	rs8118905	20	49610597	2.00E-08	1	rs8118905
136	116	20:60865815:A:G	rs13044225	20	60865815	9.00E-09	1	rs13044225
137	117	22:41209304:A:G	rs138321	22	41209304	2.00E-09	1	rs138321
138	118	22:42571028:A:G	rs760648	22	42571028	3.00E-09	1	rs760648

Moreover, after activating the GENE2FUNC function, the result was a collection of heat maps. These maps depicted the expression levels of genes across 11 distinct developmental stages of the brain, providing valuable insight into gene expression. Genes such as HLA-B, HLA-C, HLA-E, HLA-A, and CNN3 were found to be highly active, as shown in **(Figure_5).**

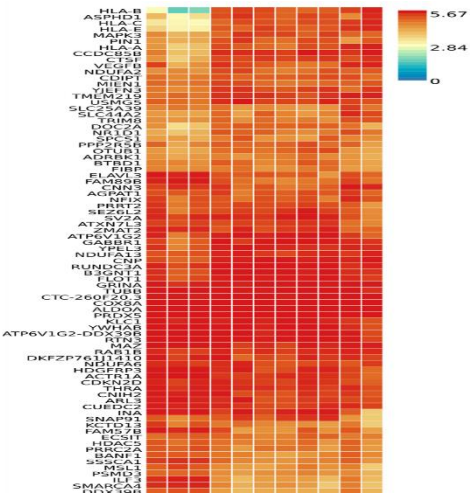


Fig. 5 | Brain Span of 11 General Developmental Stages of Brain Samples of BD via Fuma GWAS

Similar results of mutual genes were yielded when generating the heatmap of 54 tissue types as shown below in **(Figure_6)**

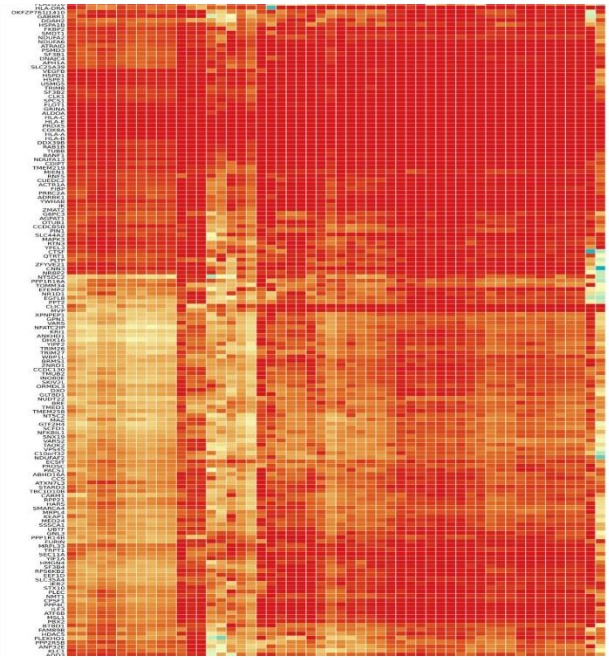


Fig. 6 | GTEx v8 54 Tissue Type of BD via Fuma GWAS

2. Functional Enrichment of Gene Sets:

DAVID was utilized to conduct GO and KEGG enrichment analysis of the genes identified previously with a significance threshold of P-value<0.05.

The biological processes analysis showed that these genes are basically associated with: antigen processing and presentation, leukocytes activation, positive regulation of T cells and interferon gamma mediated signaling pathways. **(Table_2).**

Table 2 | Biological Processes (BP) of Mapped Genes for BD via DAVID

Term	Count	P-Value	Genes
GO:0002476~antigen processing and presentation of endogenous peptide antigen via MHC class Ib	8	3.24E-08	**HLA-B**
GO:0002504~antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	8	1.22E-07	**HLA-DMA**
GO:0019882~antigen processing and presentation	10	3.99E-07	**HLA-DMA**
GO:0001916~positive regulation of T cell mediated cytotoxicity	9	7.56E-07	**HLA-B**

GO:0045321~leukocyte activation	6	3.12E-06	**HLA-B**
GO:0019886~antigen processing and presentation of exogenous peptide antigen via MHC class II	8	4.24E-06	**HLA-DMA**
GO:0002503~peptide antigen assembly with MHC class II protein complex	6	2.23E-05	**HLA-DMA**
GO:0050870~positive regulation of T cell activation	7	7.65E-05	**HLA-DMA**
GO:0042270~protection from natural killer cell mediated cytotoxicity	4	1.87E-04	**HLA-B**
GO:0060333~interferon-gamma-mediated signaling pathway	6	2.92E-04	**HLA-B**
GO:0050778~positive regulation of immune response	6	0.001104	**HLA-DMA**

As for cellular components analysis, the screened genes are localized in membranes of endoplasmic reticulum and golgi apparatus, beside the MHC II protein complex as shown in **(Table_3).**

Table 3 | Cellular Components (CC) of Mapped Genes for BD via DAVID

Term	Count	P-Value	Genes
GO:0071556~integral component of lumenal side of endoplasmic reticulum membrane	11	2.58E-10	**HLA-DRB5**
GO:0098553~lumenal side of endoplasmic reticulum membrane	11	2.58E-10	**HLA-DRB5**
GO:0012507~ER to Golgi transport vesicle membrane	12	2.96E-08	**HLA-DRB5**
GO:0042613~MHC class II protein complex	9	4.67E-08	**HLA-DMA**
GO:0042612~MHC class I protein complex	6	5.20E-07	**HLA-B**
GO:0005654~nucleoplasm	115	0.001117	**MDC1**
GO:0005739~mitochondrion	50	0.001335	**NDUFA13**
GO:0005789~endoplasmic reticulum membrane	41	0.001437	**TMEM151A**
GO:0009897~external side of plasma membrane	22	0.001901	**F10**
GO:0000139~Golgi membrane	28	0.002112	**RTN3**

In terms of molecular functions, the screened genes displayed a significant enrichment in the binding of various molecular compounds and enzymes, such as protein, receptors, peptide-antigen, TAP, manganese ion, ATP, T cell receptor, beta 2 micro-globulin and MHC II protein complex binding and receptor activity. **(Table_4).**

Table 4 | Molecular Function (MF) of Mapped Genes for BD via DAVID

Term	Count	P-Value	Genes
GO:0042605~peptide antigen binding	14	3.35E-11	**HLA-DRB5**
GO:0032395~MHC class II receptor activity	6	6.54E-06	**HLA-DMA**
GO:0023026~MHC class II protein complex binding	6	3.77E-04	**HLA-DMA**
GO:0005102~receptor binding	22	5.15E-04	**BTN3A1**
GO:0005515~protein binding	320	5.43E-04	**PNMT**
GO:0046977~TAP binding	3	0.001521	**HLA-B**
GO:0042608~T cell receptor binding	4	0.001676	**HLA-DRA**
GO:0030145~manganese ion binding	7	0.005476	**FEN1**
GO:0004867~serine-type endopeptidase inhibitor activity	8	0.011633	**WFDC12**
GO:0030881~beta-2-microglobulin binding	3	0.013162	**HLA-A**
GO:0005524~ATP binding	48	0.023865	**DDR1**

Furthermore, KEGG enrichment analysis showed that pathways are associated with: Type I diabetes, allograft rejection, antigen processing and presentation, autoimmune thyroid disease and Epstein-Barr virus infection. **(Table_5).**

Table 5 | KEGG Pathways of Mapped Genes for BD via DAVID

Term	Count	P-Value	Genes
hsa04940: Type I diabetes mellitus	13	7.72E-11	**HLA-DRB5**
hsa05330: Allograft rejection	11	5.83E-09	**HLA-DMA**
hsa05332: Graft-versus-host disease	11	1.68E-08	**HLA-DMA**
hsa04612: Antigen processing and presentation	13	1.11E-07	**HLA-DRB5**
hsa05320: Autoimmune thyroid disease	11	1.81E-07	**HLA-DMA**
hsa05416: Viral myocarditis	11	6.11E-07	**HLA-DMA**
hsa04145: Phagosome	16	1.13E-06	**HLA-DRB5**
hsa05166: Human T-cell leukemia virus 1 infection	17	2.91E-05	**HLA-DRB5**
hsa04514: Cell adhesion molecules	12	7.09E-04	**SPN**
hsa05169: Epstein-Barr virus infection	13	0.001648	**RBPJL**
hsa05203: Viral carcinogenesis	13	0.001791	**RBPJL**

3. PPI Network Construction & Analysis:

The network built by **STRING** - after defining high confidence score of 0.9 - contained 504 nodes, 210 edges, (0. 0.833) as average node degree,

(0.242) as average local clustering coefficient, and the PPI enrichment P-value < 1.0e-16. (**Figure_7)**

The anticipated number of edges between proteins is 111. Yet, the completed network shows a higher number of interactions, suggesting that the screened proteins are more interconnected with each other than random proteins of similar size and degree distribution from the genome. This finding suggests a feasible biological connection among the proteins as a cohesive unit.

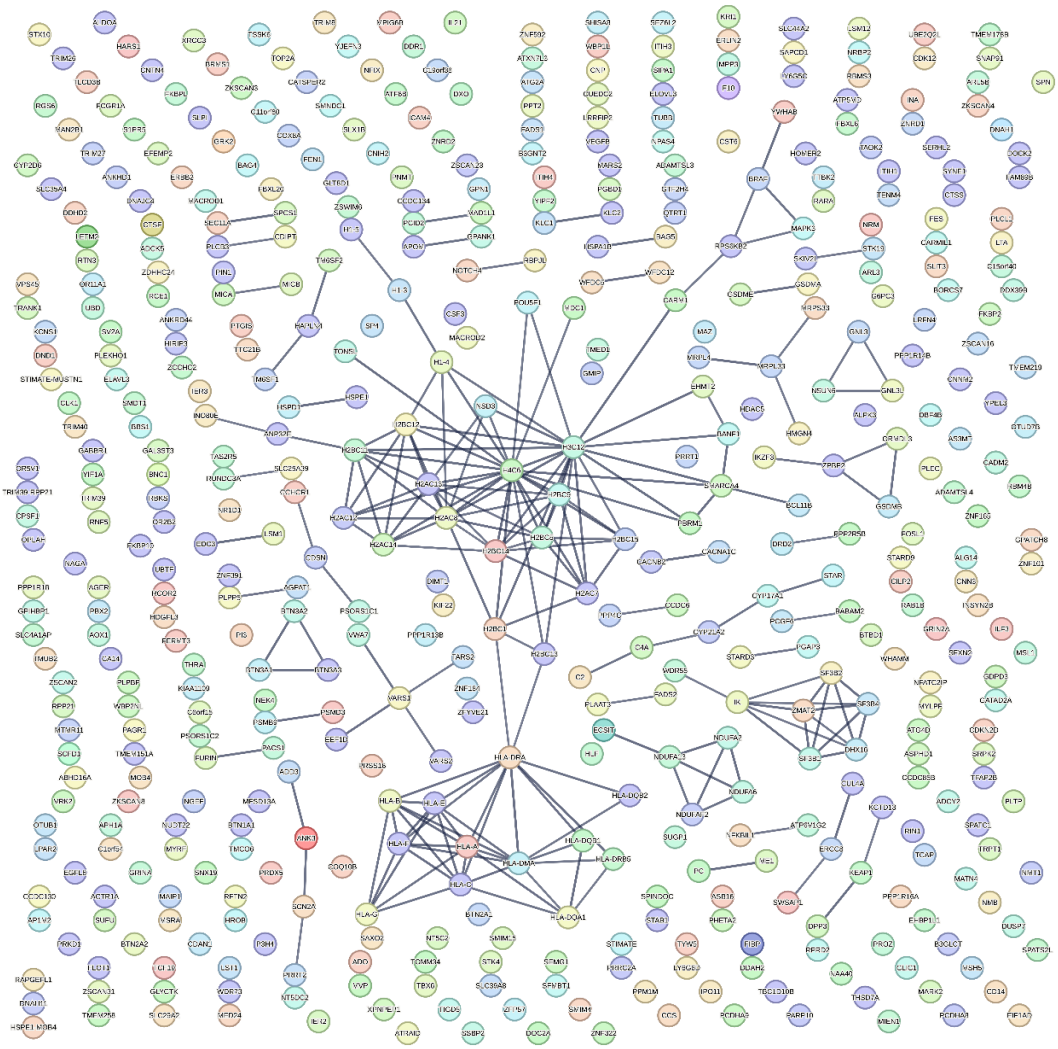


Fig. 7 | PPI Network of Mapped Genes for BD via STRING

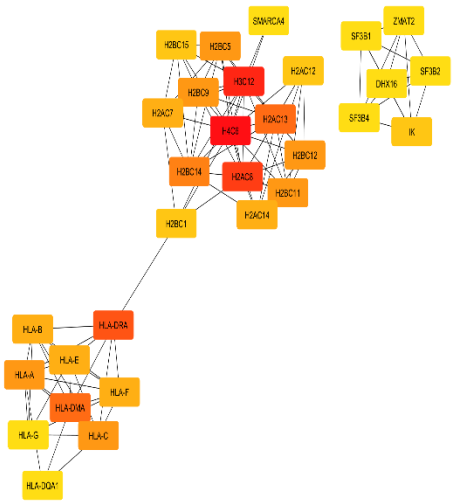
4. PPI Network Module Analysis & Hub Genes Selection:

The implementation of the CytoHubba plugin allowed the computation of connectivity scores and identification of common genes from the top 30

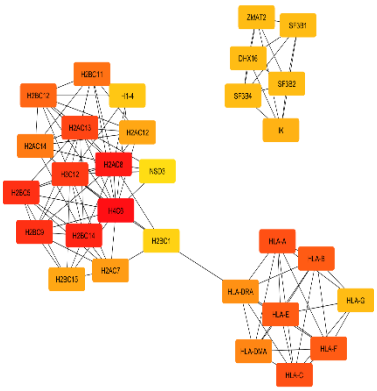
genes using a combination of four algorithms: Degree, MCC, Stress, and Radiality. (**Figure_8), (Table_6)**

Table 6 | Top 30 Hub Genes of BD Rank in CytoHubba by 4 Different Methods

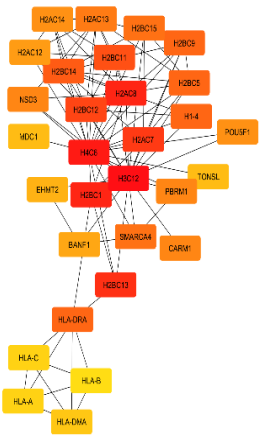
Local-Based Methods		Global-Based Methods	
DEGREE	**MCC**	**RADILITY**	**STRESS**
H2BC12	H2BC12	H2BC1	H2BC1
H2BC9	H2BC9	HLA-B	HLA-B
H2BC1	H2BC1	H2BC9	H2BC9
HLA-B	HLA-B	H2BC12	H2BC12
SF3B2	SF3B2	BANF1	H2AC7
DHX16	DHX16	H2AC7	CARM1
SF3B1	SF3B1	H2AC12	HLA-A
H2AC7	H2AC7	CARM1	HLA-C
IK	IK	HLA-A	SMARCA4
H2AC12	H2AC12	HLA-C	H2AC8
SMARCA4	HLA-A	SMARCA4	H2BC13
HLA-A	HLA-C	H2AC8	H2BC5
HLA-C	H2AC8	H2BC13	H2AC13
H2AC8	H2AC14	PBRM1	HLA-DQA1
H2AC14	H2BC5	EHMT2	RPS6KB2
H2BC5	H2AC13	H2AC14	H2BC14
H2AC13	H2BC14	H2BC5	ANP32E
HLA-DQA1	H3C12	H2AC13	H3C12
H2BC14	HLA-DRA	H2BC14	HLA-DRA
H3C12	HLA-F	H3C12	HLA-DRB5
HLA-DRA	H2BC15	HLA-DRA	HLA-F
HLA-F	H2BC11	H2BC15	BRAF
H2BC15	HLA-DMA	HLA-DMA	HLA-DMA
H2BC11	H1-4	H2BC11	HLA-DQB1
HLA-DMA	H4C6	H1-4	H2BC11
H4C6	HLA-E	H4C6	H1-4
HLA-E	HLA-G	TONSL	H4C6
HLA-G	SF3B4	POU5F1	HLA-E
SF3B4	NSD3	NSD3	H1-3
ZMAT2	ZMAT2	MDC1	NSD3



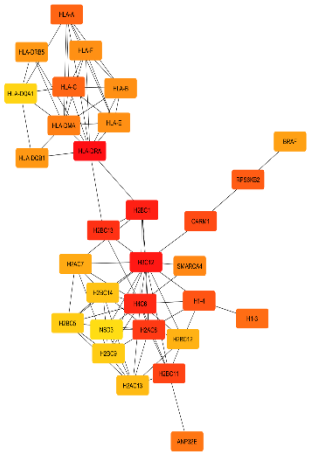
(A) Degree



(B) MCC



(C) Radility



(D) Stress

Fig. 8 | Hub Gene Networks Identified from the PPI Network of BD; Using (A) Degree Algorithm; (B) MCC Algorithm; (C) Radiality Algorithm; and (D) Stress Algorithm of the Cytoscape Plug-in CytoHubba

Among the top 30 hub genes, the 4 methods pinpointed a set of 16 central hub genes that were shared amongst them: H2AC13, H2AC7, H2AC8, H2BC1, H2BC11, H2BC12, H2BC14, H2BC5, H2BC9, H3C12, H4C6, HLA-A, HLA-B, HLA-C, HLA-DMA and HLA-DRA. These common hub genes were identified using **Venny (2.1).** (**Figure_9)**

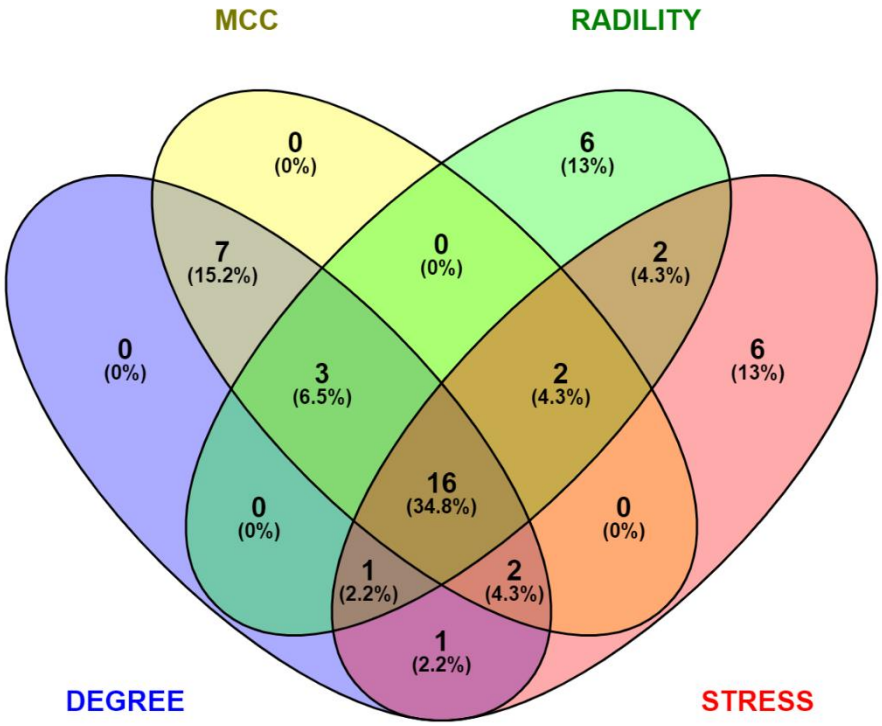
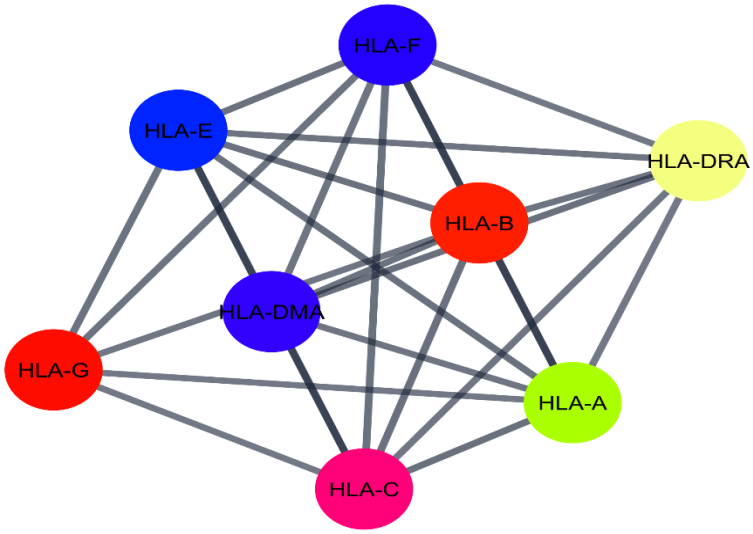
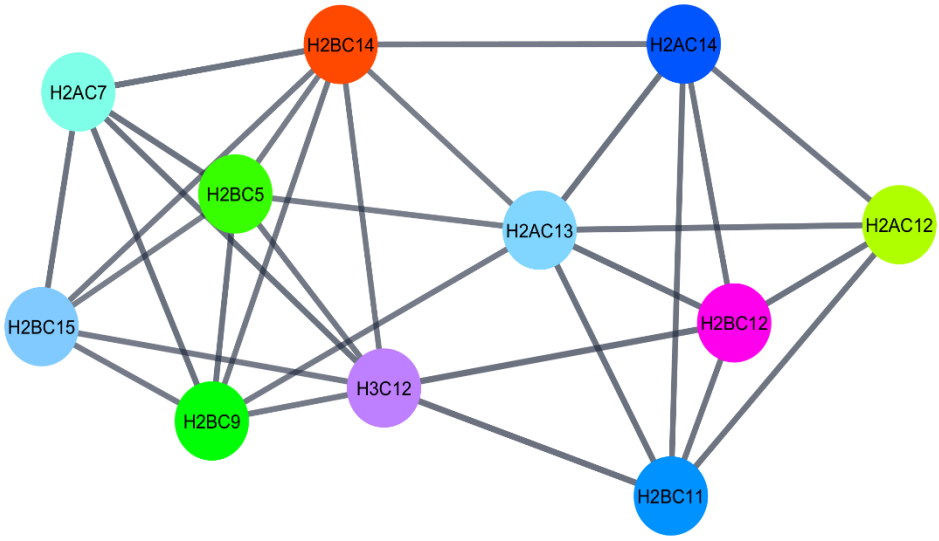


Fig. 9 | Venn Diagram of the Top 30 Genes of BD in 4 Classification Methods of CytoHubba via Venny

As **MCODE**-plugin was employed, two significant modules were defined from the PPI network with a criteria of selection as follows: degree cut-off = 2, node score cut-off = 0.2, k-score = 2, and Max depth = 100 **(Figure_10), (Table_7).**



(A) Cluster 1: Score = 7.429



(B) Cluster 2: Score = 6.2

Fig. 10 | The Most Important Modules of BD Generated by MCODE: (A) Cluster 1, (B) Cluster 2

Table 7 | Top 2 Clusters of BD Identified by MCODE

Cluster Number	Score	Nodes	Edges	Genes
1	**7.429**	8	26	HLA-A, HLA-B, HLA-C, HLA-A-E, HLA-A-F, HLA-G, HLA-DMA, HLA-DRA
2	**6.2**	11	31	H2AC13, H2AC7, H2AC8, H2BC1, H2BC11, H2BC12, H2BC14, H2BC5, H2BC9, H3C12, H4C6

In order to confirm the representation of all sixteen designated hub genes within the most highly significant modules, a thorough overlap analysis was performed. This comparison- conducted using Venny (2.1) - revealed the presence of thirteen key genes. **(Figure_11).**

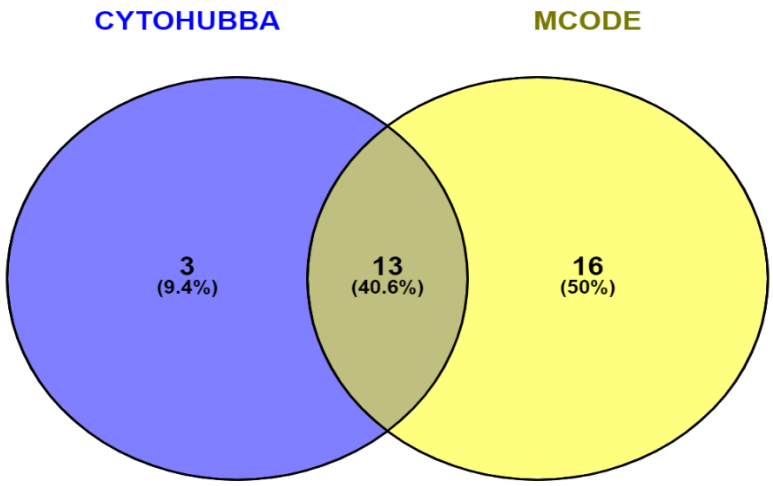


Fig. 11 | Intersection of the Hub Genes and MCODE Top 2 Clusters of BD Genes via Venny

5. Validation of Hub Genes:

The intersection analysis between hub genes and **BD** using **CTD** revealed that fifteen hub genes were linked to **BD**. **(Table_8). (Figure_12).**

Table 8 | Hub Genes Inferred Association to BD via CTD

Gene	Description	Inference Score
HLA-B	major histocompatibility complex, class I, B	**32.87**
HLA-A	major histocompatibility complex, class I, A	**28.73**
H2BC12	H2B clustered histone 12	**14.88**
H3C12	H3 clustered histone 12	**16.32**
H2BC5	H2B clustered histone 5	**11.59**
HLA-DRA	major histocompatibility complex, class II, DR alpha	**6.98**
H2BC9	H2B clustered histone 9	**6.71**
H2BC14	H2B clustered histone 14	**6.56**
HLA-C	major histocompatibility complex, class I, C	**6.43**
HLA-DMA	major histocompatibility complex, class II, DM alpha	**6.39**
H2BC1	H2B clustered histone 1	**5.19**
H2AC13	H2A clustered histone 13	**4.86**
H2BC11	H2B clustered histone 11	**4.36**
H2AC8	H2A clustered histone 8	**4.13**
H4C6	H4 clustered histone 6	**2.13**
H2AC7	H2A clustered histone 7	**-**

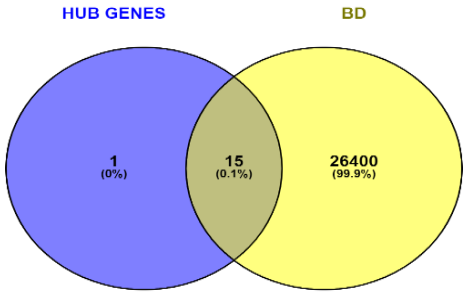


Fig. 12 | The Venn diagram of Hub Genes and BD-Inferred Genes Generated by CTD via Venny

6. Hub Genes Functional Enrichment Analysis

Using **Metascape** to visualize functional enrichment of hub genes, it showed that the hub genes are basically associated with systemic lupus erythematosus, allograft rejection, RMTs methylate histone arginines, protein loacalization to CENP-A containing chromatin, antibacterial humoral response, regulation of hematopoiesis and chromosome organization. **(Figure_13)**, **(Figure_14)**

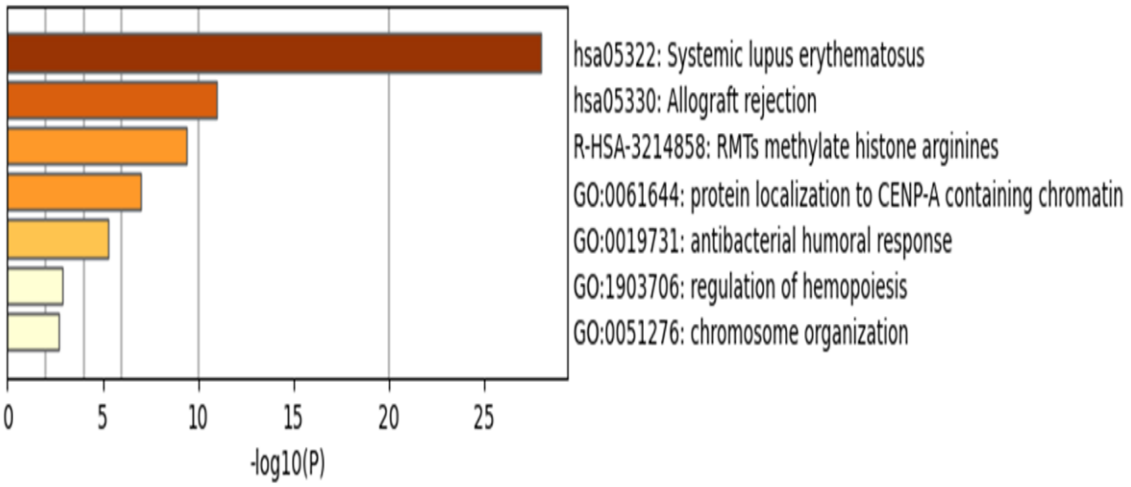


Fig. 13 | Bar Graph of Enriched Pathways and Biological Processes of the 16 Hub Genes of BD Identified by CytoHubba, Colored by P-values via Metascape

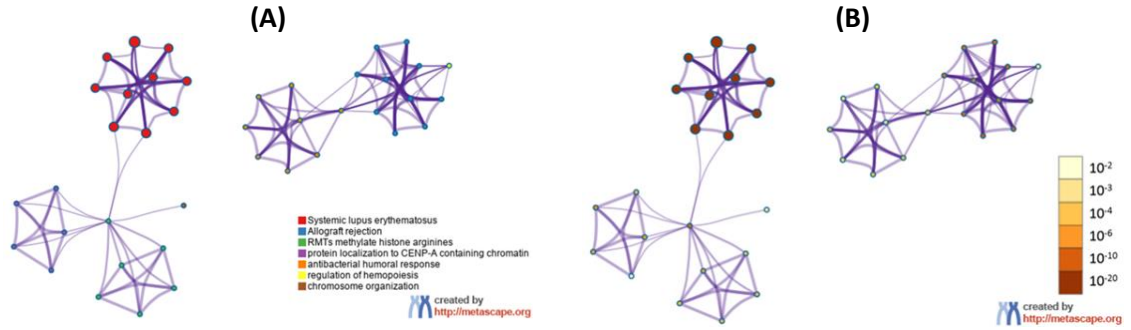


Fig. 14 | Network of Enriched Pathways and Biological Processes of the 16 Hub Genes of BD: (A) Colored by Cluster ID, Where Nodes That Share the Same Cluster ID Are Typically Close To Each Other; (B) Colored by p-value, Where Terms Containing More Genes Tend to Have a More Significant P-Value via Metascape

As for DAVID utilization, its functional annotation revealed two clusters of association. The first cluster with an enrichment score of (10.01) showed associations of H2AC13, H2AC7, H2AC8, H2BC1, H2BC11, H2BC12, H2BC14, H2BC5, H2BC9, H3C12 and H4C6 genes with ribosylation, viral carcinogenesis, protein and DNA binding, histone fold, systemic lupus erythematosus and alcoholism. **(Figure_15)**

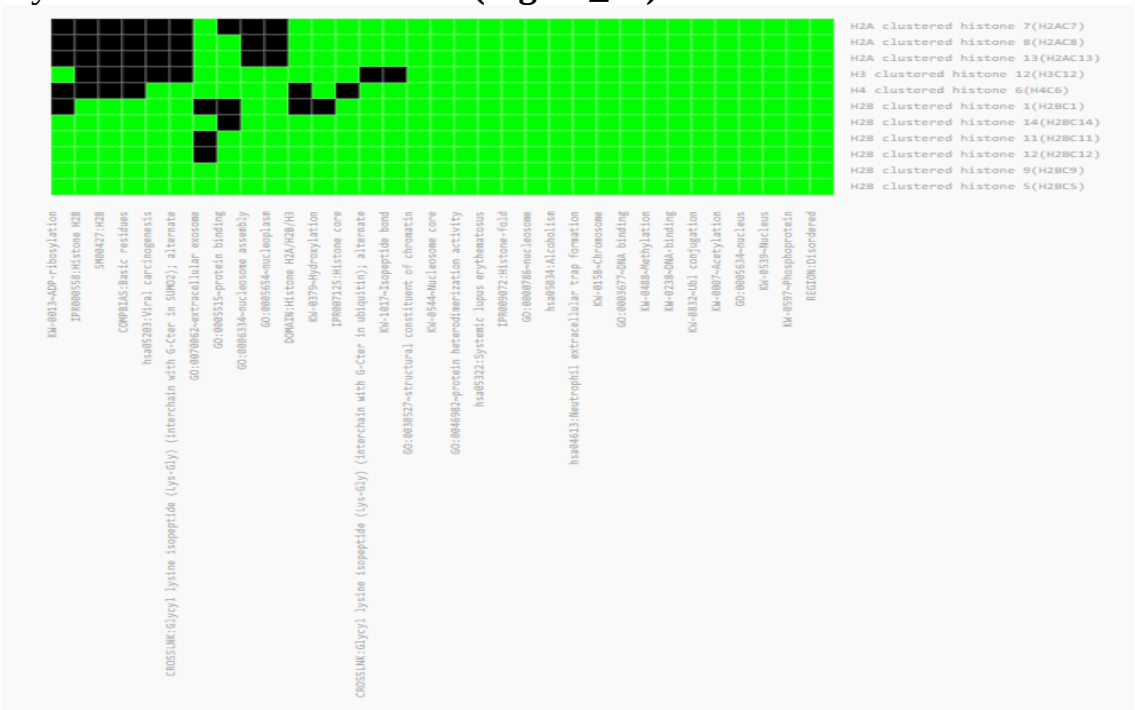


Fig. 15 | 2D View Functional Annotation Clustering Heatmap of cluster 1 of Hub Genes Associated with BD Generated by DAVID. (Green squares correspond to gene term association positively reported, while black squares correspond to gene term association not reported yet)

As for the second cluster with an enrichment score of (3.7) involving the genes; HLA-A, HLA-B, HLA-C, HLA-DMA and HLA-DRA, it revealed associations with MHC class I and II receptor activity, TAP binding, immunity and viral infections. **(Figure_16)**

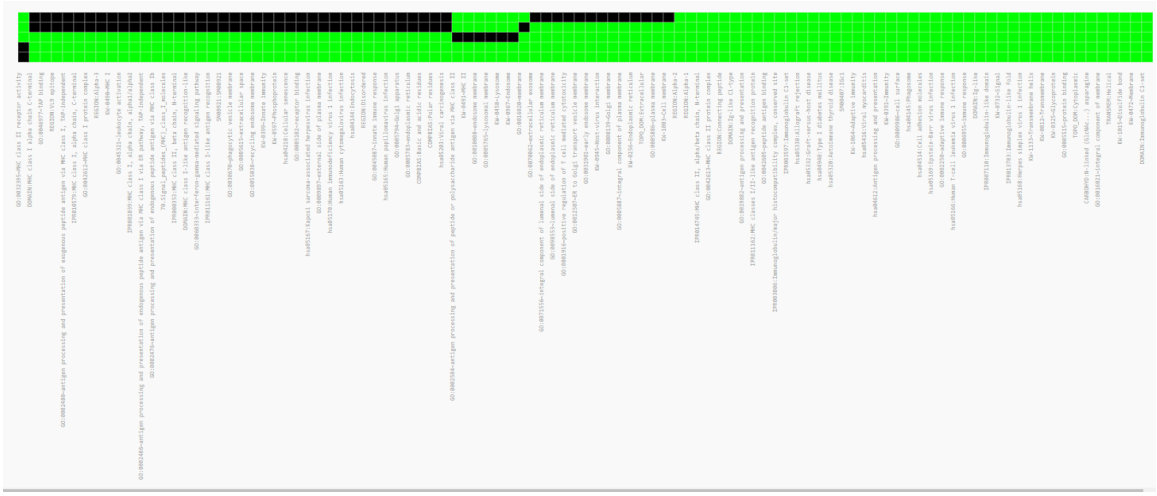


Fig. 16 | 2D View Functional Annotation Clustering Heatmap of cluster 2 of Hub Genes Associated with BD Generated by DAVID. (Green squares correspond to gene term association positively reported, while black squares correspond to gene term association not reported yet)

7. Prediction of Target miRNAs:

After uploading the sixteen hub genes to **miRTarBase** through **Enrichr**, we utilized **NetworkAnalyst** to unveil a dynamic network consisting of 762 miRNAs and 1089 edges. This allowed us to gain a comprehensive understanding of the complex interactions within the network. **(Figure_17).**

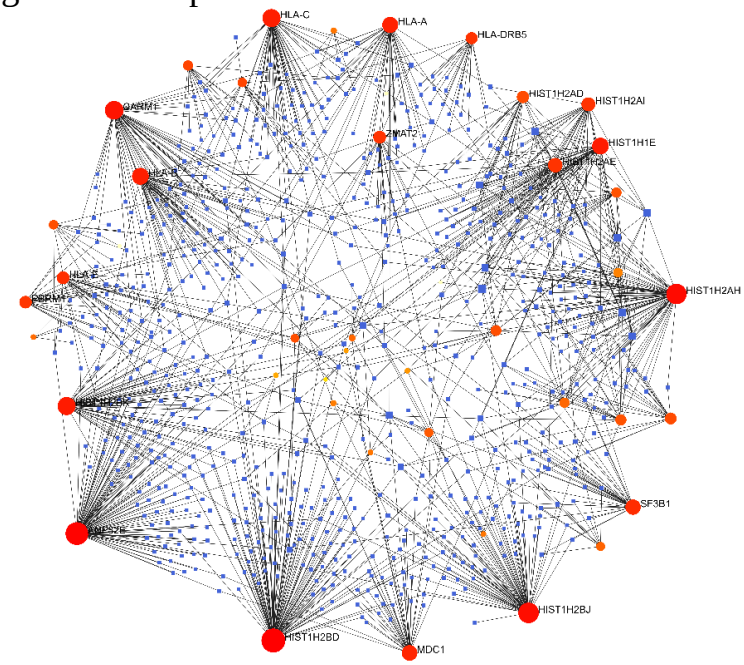


Fig. 17 | miRNA- Hub Gene Regulatory Network Associated with BD; Red Circles Represent Hub Genes while blue squares Represent miRNA via miRTarBase

Upon overlap analysis of the 762 miRNAs from miRTarBase and the 76 miRNAs from PubMed, 34 miRNAs identified in our study were found to share differential expression patterns with those previously confirmed to be associated with BD.

8. Prediction of Target Transcription Factors (TFs):

Using integrated **JASPAR** in **miRNnet** to identify the targeted transcription factors, we obtained a network consisting of 28 transcription factors with 42 edges incorporating five hub genes. **(Figure_18), (Table_9).**

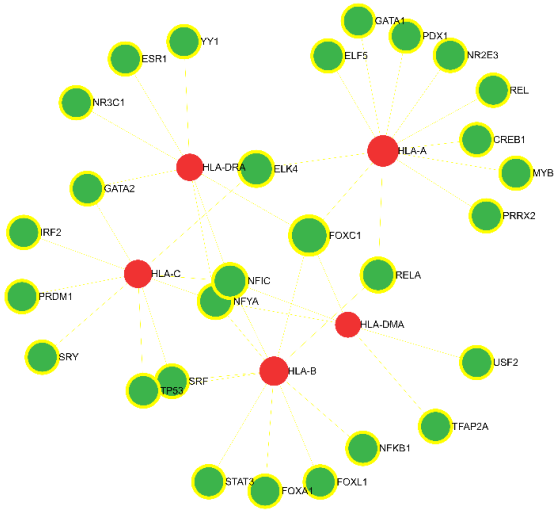


Fig. 18 | TF- Hub Gene Regulatory Network Associated with BD; Red Circles Represent Hub Genes while Green Circles Represent TF via JASPAR

Table 9 | TF & Targeted Hub Genes of BD

TF	Description	Targeted Hub Gene
FOXC1	**Fork head box C1**	**HLA-A, HLA-B, HLA-DMA, HLA-DRA**
NFIC	**nuclear factor I C**	**HLA-B, HLA-C, HLA-DMA, HLA-DRA**
NFYA	**nuclear transcription factor Y subunit alpha**	**HLA-B, HLA-C, HLA-DMA, HLA-DRA**
GATA2	**GATA binding protein 2**	**HLA-C, HLA-DRA**
SRF	**serum response factor**	**HLA-B, HLA-C**
RELA	**RELA proto-oncogene, NF-kB subunit**	**HLA-A, HLA-B**
TP53	**tumor protein p53**	**HLA-B, HLA-C**
ELK4	**ETS transcription factor ELK4**	**HLA-A, HLA-C**
ESR1	**estrogen receptor 1**	**HLA-DRA**

FOXA1	**Fork head box A1**	**HLA-B**
NR3C1	**nuclear receptor subfamily 3 group C member 1**	**HLA-DRA**
NR2E3	**nuclear receptor subfamily 2 group E member 3**	**HLA-A**
GATA1	**GATA binding protein 1**	**HLA-A**
MYB	**MYB proto-oncogene, transcription factor**	**HLA-A**
ELF5	**E74 like ETS transcription factor 5**	**HLA-A**
NFKB1	**nuclear factor kappa B subunit 1**	**HLA-B**
STAT3	**signal transducer and activator of transcription 3**	**HLA-B**
TFAP2A	**transcription factor AP-2 alpha**	**HLA-DMA**
FOXL1	**Fork head box L1**	**HLA-B**
REL	**REL proto-oncogene, NF-kB subunit**	**HLA-A**
SRY	**sex determining region Y**	**HLA-C**
PDX1	**pancreatic and duodenal homeobox 1**	**HLA-A**
PRRX2	**paired related homeobox 2**	**HLA-A**
CREB1	**cAMP responsive element binding protein 1**	**HLA-A**
YY1	**YY1 transcription factor**	**HLA-DRA**
USF2	**upstream transcription factor 2, c-fos interacting**	**HLA-DMA**
IRF2	**interferon regulatory factor 2**	**HLA-C**
PRDM1	**PR/SET domain 1**	**HLA-C**

9. Examining the Interaction Between TFs and miRNAs:

A network of 393 miRNA, 28 TF, 5 hub genes with 643 edges was obtained when examining the interaction between miRNA, TF and hub genes utilizing **JASPAR** and **miRNet**. **(Figure_19).**

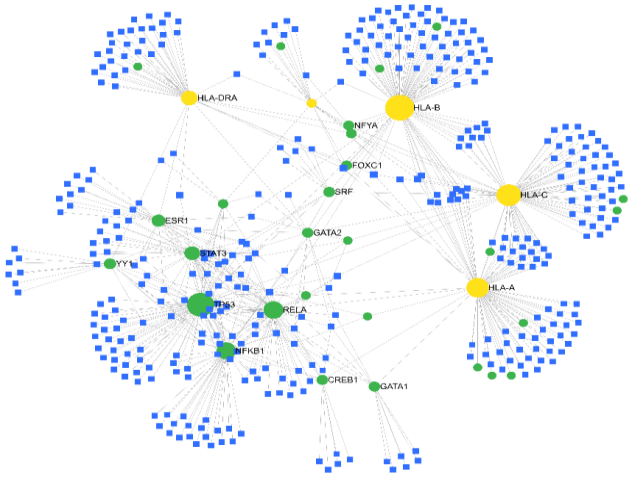


Fig. 19 | TF, miRNA and Hub Gene Regulatory Network Associated with BD via JASPAR & miRNet; Yellow Circles Represent Hub Genes while Green Circles Represent TF and Blue Squares Represent miRNA.

10. Analysis of Gene-Disease Association:

Using **DisGeNET DB** integrated in NetworkAnalyst to identify associated diseases with hub genes, three sub-networks were identified; the first one consisted of three central hub genes: HLA-A, HLA-B and HLA-C, associated with these top ten diseases: Schizophrenia, Glioma, Stevens-Johnson Syndrome, ankylosing spondylitis, Photophobia, Photodysphoria, Inflammatory abnormality of the eye, Chemical and Drug Induced Liver Injury, HIV Infections and Adverse reaction to drugs. **(Figure_20).**

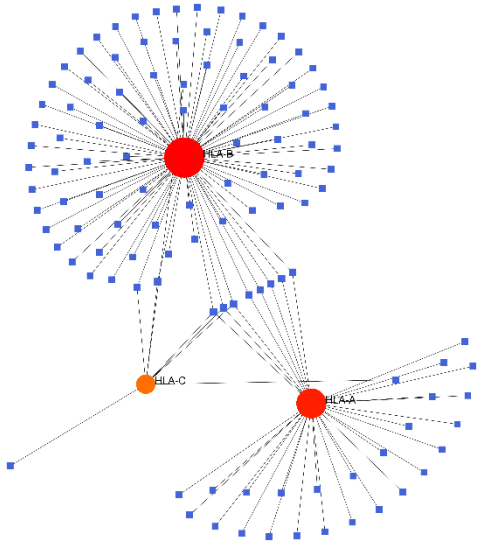


Fig. 20 | Gene-Disease Association of BD Sub-Network (1) Obtained from NetworkAnalyst: Red Circles Represent Hub Genes while Blue Squares Represent Associated Diseases via DisGeNet

As for the second sub-network, the central hub gene was HLA-DRA associated with diseases shown in (**(Figure_21).**

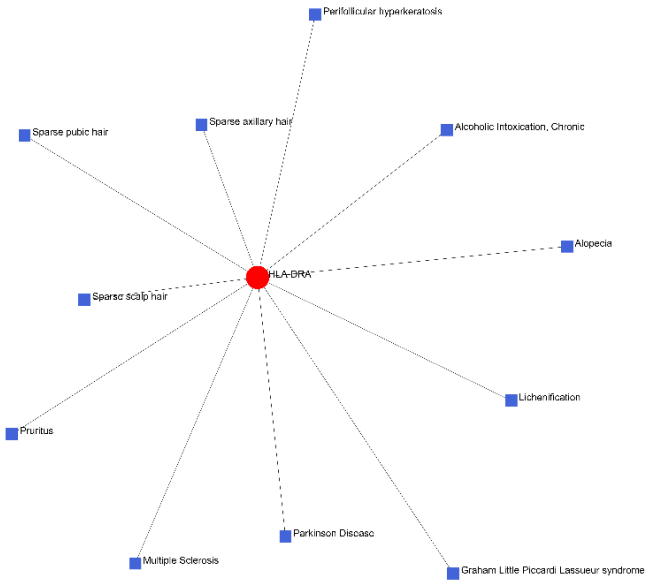


Fig. 21 | Gene-Disease Association of BD Sub-Network (2) Obtained from NetworkAnalyst: Red Circles Represent Hub Genes while Blue Squares Represent Associated Diseases via DisGeNet

At last, sub-network three identified HLA-DMA as the central gene associated with both contact and occupational dermatitis **(Figure_22).**

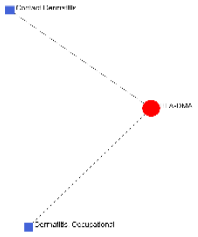


Fig. 22 | Gene-Disease Association of BD Sub-Network (1) Obtained from NetworkAnalyst: Red Circles Represent Hub Genes while Blue Squares Represent Associated Diseases via DisGeNet

By using **Metascape**, we were able to gain further understanding of BD-associated diseases. A bar graph was generated, highlighting the diseases that showed strong associations, including: hypersensitive syndrome, leishmaniasis, uveitis, drug induced stevens johnson syndrome, trachoma among others. **(Figure_23).**

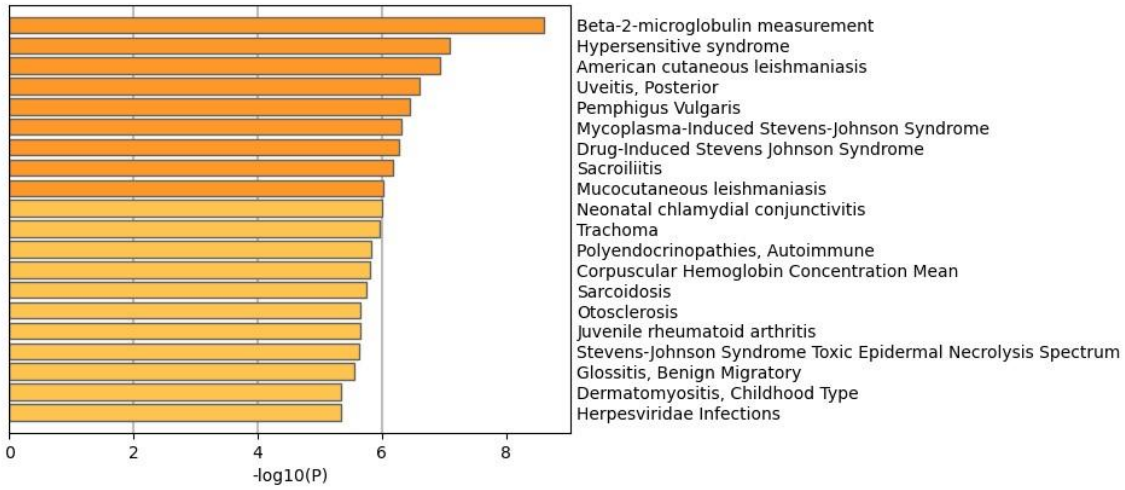


Fig. 23 | Summary of Enrichment Analysis of BD in DisGeNET via Metascape

When utilizing Coremine Medical to explore associated diseases, it showed the relevance between BD and other psychiatric disorders like; schizophrenia, borderline personality, major depressive disorder, psychotic disorders and many others as shown in **(Figure_24).**

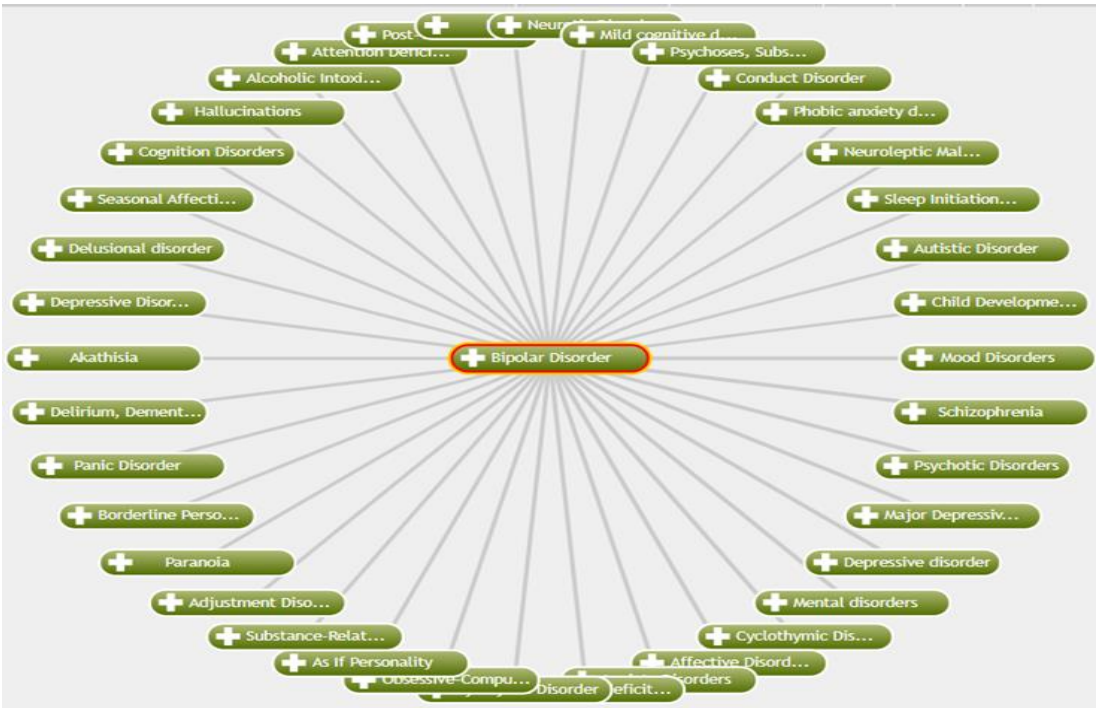


Fig. 24 | Associated Diseases with BD via Coremine Medical

11. Analysis of Candidate Drugs:

With the utilization of **DGIdb**, we successfully identified potential drugs and discovered 81 interactions. We specifically targeted the drugs relevant to BD and highlighted them in our findings. **(Table_10).**

Table 10 | Candidate Drugs for BD via DGIdb

GENE	DRUG	Regulatory Approval	Indication	Interaction Score
HLA-A	DESIPRAMINE	Approved	Tricyclic, Antidepressive Agents	0.074469765
HLA-A	TRIMIPRAMINE	Approved	Tricyclic, Antidepressive Agents	0.089363718
HLA-A	CLOMIPRAMINE	Approved	Tricyclic, Antidepressive Agents	0.078850339
HLA-A	DOXEPIN	Approved	hypnotic, antimigraine agent	0.065388086
HLA-A	CARBAMAZEPINE	Approved	for treatment of bipolar disorder	0.055852324
HLA-B	CLOZAPINE	Approved	Antipsychotic Agents	0.050067957
HLA-B	CARBAMAZEPINE	Approved	for treatment of bipolar disorder	0.097006668

Moreover, through the use of **Coremine Medical**, we were able to obtain a network which revealed the drugs that were prescribed, with the aim of validating our results **(Figure_25)**.

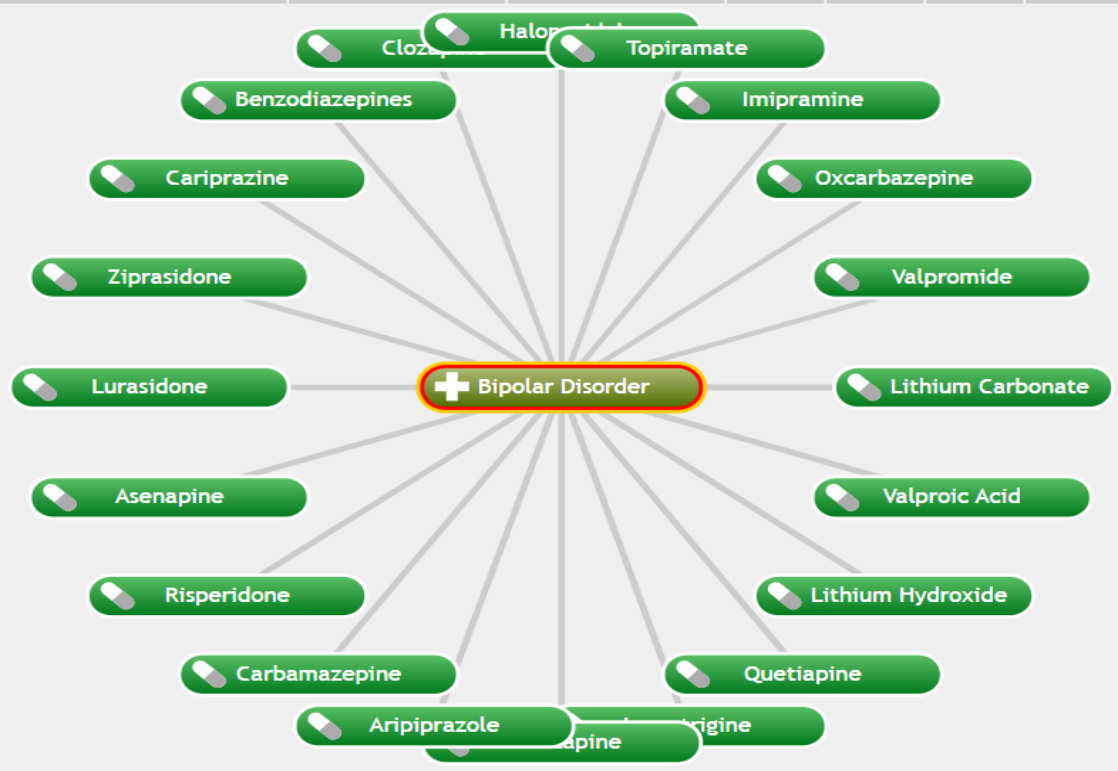


Fig. 25 | Potential Treatments of BD via Coremine Medical

In our utilization of **DGIdb** and subsequent research on transcription factors and their related drug interactions, we made a significant discovery. Not only did we uncover additional medications linked to BD, we also uncovered new associations with other previously revealed associated diseases. Our findings can be found in **(Table_11).**

Table 11 | Candidate Drugs for BD Targeting TF via DGIdb

GENE/ TF	DRUG	Regulatory Approval	Indication	Interaction Score
EHMT2	BUSPIRONE	Approved	Anti-anxiety Agents	0.01147
TP53	AMOXAPINE	Approved	antidepressant	0.011045
TP53	NORTRIPTYLINE	Approved	antidepressant	0.004909
CREB1	CITALOPRAM	Approved	antidepressant	1.382345
NR3C1	MIFEPRISTONE	Approved	antidepressant, antipsychotic	0.073725
EHMT2	MIFEPRISTONE	Approved	antidepressant, antipsychotic	0.002753
EHMT2	CITALOPRAM	Approved	antidepressant	0.002151
EHMT2	PHENELZINE	Approved	Antidepressive Agents	0.008603
TP53	TRIFLUOPERAZINE	Approved	Antiemetics; Antipsychotic Agents	0.015905
TP53	TRIFLUPROMAZINE	Approved	Antiemetics; Antipsychotic Agents	0.016567
EHMT2	TRIFLUOPERAZINE	Approved	Antiemetics; Antipsychotic Agents	0.002753
EHMT2	RESERPINE	Approved	Antihypertensive Agents; Antipsychotic Agents	0.002868
EHMT2	ZIPRASIDONE	Approved	antipsychotic agent	0.003277
EHMT2	PALIPERIDONE	Approved	antipsychotic agent	0.006257
TP53	HALOPERIDOL	Approved	Antipsychotic Agents	0.004275
TP53	PERPHENAZINE	Approved	Antipsychotic Agents	0.006627
TP53	FLUPHENAZINE	Approved	Antipsychotic Agents	0.008836
TP53	THIORIDAZINE	Approved	Antipsychotic Agents	0.006025
EHMT2	MOLINDONE	Approved	Antipsychotic Agents	0.017205
EHMT2	FLUSPIRILENE	Approved	Antipsychotic Agents	0.004048
EHMT2	THIORIDAZINE	Approved	Antipsychotic Agents	0.003128
EHMT2	FLUPHENAZINE	Approved	Antipsychotic Agents	0.004588
EHMT2	MESORIDAZINE	Approved	Antipsychotic Agents	0.017205
NR3C1	CARBAMAZEPINE	Approved	for treatment of bipolar disorder	0.0096
PBRM1	ALPRAZOLAM	Approved	hypnotic, sedative, anxiolytic	0.317097
NFKB1	PROMETHAZINE	Approved	Hypnotics and Sedatives; Anti-anxiety agents; Anti-allergic Agents	0.029564
EHMT2	PROMETHAZINE	Approved	Hypnotics and Sedatives; Anti-anxiety agents; Anti-allergic Agents	0.003277

PBRM1	TRIAZOLAM	Approved	sedative, analgesic	0.327667
TP53	CLOMIPRAMINE	Approved	Tricyclic, Antidepressive Agents	0.003898
NFKB1	PROTRIPTYLINE	Approved	Tricyclic, Antidepressive Agents	0.088692
NFKB1	PROTRIPTYLINE	Approved	Tricyclic, Antidepressive Agents	0.088692
EHMT2	CYCLOBENZAPRINE	Approved	Tricyclic, Antidepressive Agents	0.007647
EHMT2	IMIPRAMINE	Approved	Tricyclic, Antidepressive Agents	0.004588
NFKB1	BACLOFEN	Approved	for treatment of alcohol dependance	0.062084
EHMT2	NALOXONE	Approved	for treatment of opioid addiction, analgesic	0.005294
EHMT2	METHYSERGIDE	Approved	Anti-migraine agents; Vasoconstrictor Agents	0.006882
BRAF	EVEROLIMUS	Approved	immunosuppressant	0.058473
ESR1	EVEROLIMUS	Approved	immunosuppressant	0.025141
PBRM1	EVEROLIMUS	Approved	immunosuppressant	0.289118
TP53	PROPYLTHIOURACIL	Approved	Antithyroid Agents	0.015593
HLA-B	CARBIMAZOLE	Approved	Antithyroid Agents	0.620843
HLA-B	PROPYLTHIOURACIL	Approved	Antithyroid Agents	0.182601
HLA-B	METHIMAZOLE	Approved	Antithyroid Agents	0.182601
HLA-B	CARBIMAZOLE	Approved	Antithyroid Agents	0.620843
HLA-B	PROPYLTHIOURACIL	Approved	Antithyroid Agents	0.182601
HLA-B	METHIMAZOLE	Approved	Antithyroid Agents	0.182601
EHMT2	METHIMAZOLE	Approved	Antithyroid Agents	0.004048
BRAF	HYDROXYCHLOROQUINE	Approved	antirheumatic agent	0.047336
ESR1	LEFLUNOMIDE	Approved	Antirheumatic Agents	0.043835
TP53	RUXOLITINIB	Approved	Anti-inflammatory agent, antineoplastic agent	0.020391
BRAF	RUXOLITINIB	Approved	Anti-inflammatory agent, antineoplastic agent	0.050977
NR3C1	HALOBETASOL	Approved	Anti-inflammatory Agents	0.307188
NR3C1	FLUOROMETHOLONE	Approved	Anti-Inflammatory Agents; Anti-allergic agents; Glucocorticoids	0.460782
NR3C1	CICLESONIDE	Approved	Anti-Inflammatory Agents; Anti-allergic agents; Glucocorticoids	0.307188
NR3C1	FLUMETHASONE PIVALATE	Approved	Anti-Inflammatory Agents; corticosteroid	0.345586
NR3C1	RIMEXOLONE	Approved	Anti-Inflammatory Agents; Corticosteroids	0.184313
NR3C1	PREDNICARBATE	Approved	Anti-Inflammatory Agents; Corticosteroids	0.230391
TP53	METHYLPREDNISOLONE	Approved	Anti-Inflammatory Agents;	0.026508

			Glucocorticoids	
NR3C1	HYDROCORTAMATE	Approved	Anti-Inflammatory Agents; Glucocorticoids	0.691173
NR3C1	DESOXIMETASONE	Approved	Anti-Inflammatory Agents; Glucocorticoids	0.184313
NR3C1	FLURANDRENOLIDE	Approved	Anti-Inflammatory Agents; Glucocorticoids	0.307188
NR3C1	PARAMETHASONE	Approved	Anti-Inflammatory Agents; Glucocorticoids	0.230391
NR3C1	METHYLPREDNISOLONE	Approved	Anti-Inflammatory Agents; Glucocorticoids	0.092156
NR3C1	DIFLORASONE DIACETATE	Approved	Anti-Inflammatory Agents; Glucocorticoids	0.184313
BRAF	CELECOXIB	Approved	NSAID	0.010195
NR3C1	PREDNISOLONE	Approved	corticosteroid, anti-inflammatory agent	0.080136
NR3C1	CLOBETASOL	Approved	corticosteroid, anti-inflammatory agent	0.115195
HLA-DQA1	INTERFERON BETA-1B	Approved	for treatment of multiple sclerosis	1.474501

The core of our investigation lies in establishing a connection between targeted hub genes and transcription factors, as well as the candidate drugs and associated diseases uncovered in this study. For instance, our gene disease analysis uncovered a comorbidity of BD with conditions such as allograft rejection, inflammatory disease, autoimmune thyroid disease and sclerosis. These findings are further supported by the presence of immunosuppressant, anti-inflammatory, and antithyroid drugs in **(Table_11)**. Moreover, our study validates the link between BD and other psychiatric disorders, including alcoholism, substance abuse, addictions, and anxiety, through the targeted hub genes and transcription factors identified.

Discussion:

This discovery of new genetic risk factors holds numerous advantages that is proved to be integral. These advantages include advancing our comprehension of the complex pathogenesis of diseases, as well as the potential to predict an individual's chances of developing them.

The classical human leukocyte antigen HLA cluster, located on chromosome 6 within the human major histocompatibility complex MHC region, is a highly diverse and gene-rich area of the human genome. It stretches over 4 Mb and contains nearly 250 genes, boasting more than 17,000 alleles according to the IMGT/HLA Database.

These genes play a crucial role in the body's adaptive immune responses, including antigen processing, intercellular recognition, and self vs. non-self-discrimination. The HLA-A, -B, and -C molecules, found in the classical HLA class I gene cluster, are particularly important in identifying and eliminating virus-infected cells, tumor cells, and transplanted allogeneic cells by producing proteins that present endogenous antigens to $CD8^+$ cytotoxic T cells and interact with natural killer (NK) cells. Furthermore, the class II molecules play a vital role in immune function by producing proteins presenting the exogenous antigens to $CD4^+$ T helper cells. As a result, HLA-class I and II play significant roles in combating infections and contributing to the emergence of autoimmune disorders.

Recent genome-wide association studies (GWAS) have confirmed the polygenic involvement in major psychiatric disorders, with a particular focus on the high statistical significance of the major histocompatibility complex (MHC) locus in schizophrenia (SZ) and, to a lesser extent, bipolar disorder (BD). These findings strongly suggest that abnormalities in immune regulation may play a role in the development of these disorders. (Tamouza et al., 2018).

Furthermore, through scientific explorations, it has been revealed that certain mental disorders; including schizophrenia, bipolar disorder, and autism, have a significant relationship with the HLA gene.

In addition, scientific research has consistently shown a correlation between bipolar disorder and specific genetic changes, particularly the HLA system which emerged as a significant factor. For instance, the HLA-B16 antigen has commonly been linked to mood disorders, such as mania and depression. Additionally, the presence of HLA-A10, HLA-A29, HLA-B7, HLA-B16, and HLA-B21 has been observed at higher rates among individuals with bipolar disorder compared to those without the condition.

Moreover, the HLA-cluster was investigated in a sample of Korean individuals, yet it failed to uncover any noteworthy associations. Similarly, a separate study conducted on Turkish Caucasians also found no correlation between HLA antigens and type I bipolar disorder. These researches findings suggest that the HLA may not play a significant role in susceptibility to the disorder in these populations, but it is also possible that the sample sizes were too small to detect any potential associations of low intensity. However, these results should not discourage further investigations in this area since the HLA genes are extremely polymorphic and can vary greatly among different ethnic groups and races, which make it possible that a link may exist in certain populations.

As recent advancements in serotyping techniques and the development of HLA genotyping have revealed a complex level of allelic polymorphism and its potential involvement in the pathogenesis of various psychiatric diseases, this progress has also greatly aided ongoing research on the association between psychiatric disorders and HLA, through the utilization of more manageable diagnostic criteria like the Diagnostic and Statistical Manual (DSM) and the International Classification of Diseases (ICD).

With these advancements, it is now possible to conduct more reliable and easily comparable studies on the potential connections between psychiatric disorders and HLA system. (Alves et al., 2006)

In another research, the impact of HLA genes on numerous health conditions has been investigated. These conditions included; autoimmune diseases and psychiatric disorders like schizophrenia and BD. Interestingly, there seems to be a connection between susceptibility to infection and autoimmunity, as well as psychiatric disorders, with evidence from both genetics and epidemiology. It has also investigated the link between specific HLA alleles and psychiatric disorders, as well as autoimmunity as a whole. (Nudel et al., 2019)

In another research, the potential connection between alleles linked to psychiatric disorders and autoimmune disease and their potential association with infections was sought. Surprisingly, there were no overlapping alleles between psychiatric disorders and infections. However, a few alleles strongly linked to autoimmune disease did show some degree of association with infections. Notably, when an allele was linked to both autoimmune disease and infection, its impact was greater on the former. Interestingly, the direction of association for HLA-C alleles in both disease classes varied, with autoimmune disease showing a protective effect while infections showed increased risk. It is possible that the latter outcome could

be clarified by examining a potential mechanism in which certain HLA-C alleles cause a decrease in immune response to particular ligands. This in turn may lower the chance of developing autoimmune disease but increase susceptibility to infection, especially if there is a structural linkage between an infectious antigen and a self-antigen that can bind to the HLA molecule. (Nudel et al., 2021b)

An important discovery has been made in this regard since these conditions have been found to have individual and combined impacts on mood disorders. Similarly, in line with other immune disorders, bipolar disorder could be better understood by considering the interplay of genes and the environment. (Avramopoulos et al., 2015)

In support of the well-established association between these HLA haplotypes and prevalent immune disorders, a research hints at the potential role of HLA-mediated pro-inflammatory pathways in BD. Immune system abnormalities, which are integral to BD, also commonly manifest as other comorbid conditions such as multiple sclerosis, thyrotoxicosis, rheumatoid arthritis, ulcerative colitis, and psoriasis. These conditions highlight the significant impact of BD on the immune system. (Eaton et al., 2010)

In regards of treatment, Lithium - a mood-stabilizing agent often used for the treatment of bipolar disorder as stated earlier- has been suggested to change the expression of HLA molecules by some researches. As the two main HLA classes seem to be affected differently by the drug; changes in class II HLA are more significant from the functional perspective, whereas changes in class II HLA have occurred at the genomic DNA level. (Kang et al., 2000)

Recent findings indicate that host immune-genetics plays a crucial role in both the development of BD and its associated clinical features. This is supported by evidence regarding the Toll-like receptor 4 (TLR-4) and (TLR-2) genes; which belong to the pattern recognition receptors family (PRR) whose activation leads to an intracellular signaling pathway and inflammatory cytokine production which is responsible of activating the innate immune system, showing that functional polymorphisms these genes are linked to the early-onset of BD and autoimmune comorbidities. Furthermore, research has revealed an additive effect of TLR-2 polymorphisms and early-life stress in increasing the risk of early-onset of BD. (Oliveira et al., 2014)

If these connections could indicate a connection between imperfect innate immune responses, persistent inflammation, and autoimmune reactions, it

would implicate the adaptive immune system, specifically the human leukocyte antigen (HLA) system. (Trowsdale & Knight et al., 2013)

As we can see, the unique characteristics of HLA genes have drawn significant attention towards their potential role in determining susceptibility to infections, autoimmune disease and psychiatric disorders across diverse populations. Consequently, a multitude of studies have investigated their involvement, resulting in an increased number of reported associations. (Blackwell et al., 2009)

Our study highlighted the potential impact of the HLA cluster on bipolar disorder, especially; HLA-A, HLA-B, HLA-C, HLA-DRA and HLA-DMA, with evidence from previous studies supporting this finding. This is further reinforced by our examination of comorbidities such as autoimmune diseases, allograft rejection, autoimmune thyroid disease, and viral carcinogenesis, as well as comorbidity with other psychiatric disorders like schizophrenia.

Over the past few years, there has been a surge in research examining the interactions of gene regulation in the field of neuroscience. Specifically, there has been a focus on epigenetic modifications, which are changes that can be inherited but also reversed. These modifications include DNA methylation, DNA hydroxyl-methylation, modifications to histones, and non-coding RNAs. Researchers have proposed a complex model called gene–environment interaction (G×E), which suggests that the development of psychiatric disorders like bipolar disorder is influenced by a combination of genetics, environmental factors, and epigenetic markers. Due to its complex nature and strong heritability, it is a particularly intriguing candidate for neurobiological investigations beyond traditional DNA sequencing methods. (Ludwig & Dwivedi et al., 2016)

Epigenetic changes refer to alterations in the function of genes that do not involve changes in the actual sequence of the gene. Recent evidence suggests that these changes can occur in cells that are actively dividing or not dividing at all, and can even be inherited across generations. These changes occur at the molecular level through modifications of nucleosomes, which is a functional unit of the genome made up of pairs of histones (H2A, H2B, H3, and H4) and a 147-bp segment of DNA, allows for the regulation of gene transcription by controlling access to the gene. While there are many types of epigenetic changes that can impact gene regulation, one of the most extensively studied in the realm of molecular psychiatry is the methylation of CpG sites in DNA. (McGowan & Kato et al., 2007b)

Studies also implicate that multiple neurological and psychiatric disorders are not caused by a singular genetic mutation, but rather involve complex disruptions in multiple genes and signals that govern their function. Recent studies have also revealed the presence of complicated epigenetic processes, which regulate gene activity without changing the actual genetic code in mature neurons. They also present current evidence supporting the presence of ongoing epigenetic mechanisms that regulate gene function in neurons which play a crucial role in complex behavior, including those associated with various psychiatric disorders such as depression, addiction, schizophrenia and BD.

Other studies suggest that the epigenetic mechanisms that have the ability to influence gene expression without changing the genetic code, may be responsible for causing persistent changes in brain function. These recent findings on epigenetic processes impact neurobiological adaptations related to enduring behaviors in animal models of psychiatric disorders as well as in individuals affected by these conditions.

Through intricate and precise mechanisms, chromatin remodeling ensures DNA accessibility to the transcriptional machinery, thereby altering gene activity without changing the underlying genetic code.

The complex process of chromatin remodeling holds important implications for both neural development and the functioning of fully mature neurons. As synaptic transmission occurs, neurons react to neurotransmitters through receptor-mediated signal transduction. This process activates or inhibits various transcription factors that play a significant role in gene regulation. The success of transcriptional activity relies on the interactions between these factors and various co-activators and co-repressors, as well as the structure of chromatin. Therefore, chromatin remodeling plays a crucial role in the activation or suppression of genes in response to synaptic activity, causing a major impact on the expression of genes as a result. (Tsankova et al., 2007)

Recent research has indicated that chromatin remodeling has also a significant impact on a variety of important processes in the brain. These include circadian rhythm, memory formation, drug addiction, and depression. In fact, studies have suggested that changes in chromatin structure via histone modification play a key role in regulating gene expression. One of the main players in this process is the enzyme histone deacetylase (HDAC), which is responsible for removing acetyl groups from specific amino acid residues. This process is crucial in the regulation of

gene expression and has been widely recognized as a fundamental mechanism in chromatin remodeling. (Hobara et al., 2010)

The growing field of epigenetic therapy involves the use of drug treatments to alter epigenetic mechanisms that control gene expression and address disease. While researchers are exploring various classes of epigenetic drugs, the majority of current investigations center on two types: those that target DNA methyltransferase (DNMTi) and those that target histone deacetylase (HDACi). Therefore, the research about the effectiveness of epigenetic drugs for mood disorders is still in its early stages. The only exception is valproic acid, which has been utilized as a mood stabilizer in clinical settings for several years and functions as a HDACi, but it's worth noting that valproic acid also has non-epigenetic actions on neuronal activity that could also play a role in its mood-stabilizing capabilities. (Peedicayil & Kumar et al., 2018).

The results of our study have highlighted the important involvement of histone genes, such as H2BC12, H3C12, H2BC5, H2BC9, H2BC14, H2BC1, H2AC13, H2BC11, H2AC8 and H4C6 in the development of BD. These findings serve as confirmation of the necessity for further investigation in this area.

This cutting-edge research on epigenetic modifications is leading to new therapeutic approaches for mood disorders. From the elucidation of the epigenetic mechanism behind successful mood stabilizers to the development of novel compounds showing promising results in preclinical trials, the potential for epigenetic interventions is expanding. For example, the use of Epi-Effectors, such as transcription activator-like effectors and zinc-finger-proteins, to target specific loci in the genome is a groundbreaking advancement. While these interventions have only been tested in animal models, they offer great potential in the field of neuroscience. However, there are concerns about their ability to affect the desired cell type and produce systemic, rather than specific, changes. To move forward, a deeper understanding of the underlying mechanisms is crucial. (Ludwig & Dwivedi et al., 2016)

In summary, research on the epigenetics of bipolar disorder is still in its early stages. While clinical genetics studies have suggested the involvement of genomic imprinting, there have been no direct tests of this hypothesis. Pharmacological studies propose the potential for manipulating DNA methylation to impact mood states, but no human experiments have been conducted so far. Recent studies have directly examined DNA

methylation in patient samples, providing promising insights into mood disorder epigenetics. (McGowan & Kato et al., 2007)
In conclusion, while still in its early stages, the emerging findings in this field hold great potential for advancing our understanding of bipolar disorder.

Limitations:

Bridging the gap between research and real-world clinical practice proves to be a major challenge, as evidenced by the struggle to effectively apply research findings. Besides, the high rate of missed diagnoses and the prolonged delay of 5 or more years before bipolar disorder is recognized as the underlying cause for mood symptoms remains a pressing issue. On the other hand, borderline personality disorder (BPD) and bipolar disorder are frequently associated with confusion and misunderstanding. Though they may both exhibit shared symptoms, it is crucial to differentiate between them as they are separate disorders with their own distinct traits. Furthermore, the reported associations between Human Leukocyte Antigen (HLA) and bipolar disorder suffer from publication bias, as a considerable portion of the studies have limited sample sizes. Finally, although the influence of epigenetics on bipolar disorder holds promise, current research in this area is still in its early stages.
As for my research limitations, due to the large number of primary variants this study was built on, processing and filtering the data was time consuming and may have been subjected to human errors. Furthermore, the restrictions to access both scientific articles and bioinformatics tools as a result of sanctions against Syria somehow hindered the time flow of the research. Besides, the time limitation was not enough to cover all the intended points.

Recommendations:

Translating research discoveries into practical application in clinical settings poses a significant challenge, as there is often a disconnection between research and its implementation. To bridge this gap, we suggest incorporating patients' perspectives into both healthcare and research practices. Furthermore, with a large number of cases going undiagnosed, it

is crucial to effectively identify bipolar disorder in a timely manner. This requires improvement in current methods. For a thorough understanding of mood swings, it is essential for mental health professionals to conduct a comprehensive evaluation, taking into account potential triggers, associated symptoms, and an individual's personal history. This is necessary to differentiate between overlapping symptoms of bipolar disorder and borderline personality disorder, allowing for appropriate and tailored treatment. In addition, further research and studies are necessary to achieve a more thorough understanding of the link between HLA genes and epigenetics in the development of BD.

Conclusion:

In conclusion, by methodically analyzing the data obtained from genome-wide association studies (GWAS) and GWAS Catalog, we were able to identify 118 genomic risk loci and 539 correlated genes. Through the integration of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, we gained a deeper understanding of the fundamental biological processes and key pathways involved in BD. As a result, we constructed a comprehensive protein-protein interaction (PPI) network, unveiling 16 central hub genes and two remarkable modules.

Through the utilization of the Comparative Toxicogenomics Database (CTD), we conducted virtual validation of the central genes. In addition, functional enrichment analysis has provided insight into the crucial roles of these key genes in biological processes such as antigen processing and presentation, as well as regulation of T-cell mediated immunity. Furthermore, our analysis uncovered 34 validated microRNAs and 28 transcription factors that target these hub genes, further establishing their significant role in BD disorder. Besides, the utilization of the DisGeNET database yielded a selection of the top ten associated diseases, including Schizophrenia, Glioma, Stevens-Johnson Syndrome, ankylosing spondylitis, Photophobia, Photodysphoria, Inflammatory abnormality of the eye, Chemical and Drug Induced Liver Injury, HIV Infections, and adverse reactions to drugs. Through the use of DGIdb, we discovered a total of nine medications that effectively treat BD: Desipramine, Trimipramine, Clomipramine, Doxepine, Carbamazepine, and Clozapine.

This research has significant implications for our understanding of the underlying molecular processes involved in BD and identifying potential targets for therapeutic interventions, by shedding light on the complex

molecular mechanisms and uncovering key biomarkers. However, it is crucial to note that the findings from this bioinformatics research must be validated through wet lab experiments. Only through further experimentation can we confirm the validity and precision of these results, leading to a more comprehensive understanding of BD development and ensuring the reliability of the identified biomarkers and therapeutic targets.

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