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USING GENOME WIDE ASSOCIATION STUDIES(GWAS)OF BIPOLAR DISORDER TO FIND A POSSIBLE TARGET/CANDIDATE GENE

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ABSTRACT

Bipolar disorder is a psychiatric disorder which affects thought, perception, emotion, and social behaviour. It consists of mania, hypomania and depression. It is broadly classified into two types namely type 1 and type 2. The aim of this study is used to find a possible target/ candidate gene for bipolar disorder using Genome Wide Association Studies (GWAS). The approach used is Gene Set Enrichment Analysis (GSEA) using algorithms such gene to function, identifying biological processes of the genes.Then clinical data related to the hypothesized was found. The hypothesized gene might be a potential target and candidate gene of bipolar disorder because of it's minimal expression in some parts of the brain.The scope of the work is it may add a gene to the list of existing genes of bipolar disorder. The limitation is further research is needed to know more about the hypothesized gene.

INTRODUCTION

Bipolar disorder (BD) is a psychiatric disorder which traits reoccurring episodes of mania, hypomania depression This mood alternating disease affect thought, perception, emotion, and social behaviour. It is a major health problem and it triggers thoughts of suicide and affects quality of life in many aspects. Based on the lifetime mood episodes, BD is classified into two main types, bipolar I disorder (BD1) and bipolar II disorder (BD2)(Smeland *et al.* 2020).In BD1, manic episodes and depressive episodes occur. In (BD2) hypomanic episode and depressive episode occur and manic episode don't occur. The population affected by bipolar disorder are Europeans, Africans. Asians, African Americans, and Latin Americans. Other population such as Koreans and Japanese are also affected by bipolar disorder. As of 2017, it was estimated that 46 million, or about 0.6 percent of the entire world population, has bipolar disorder (Kessler *et al.*2005).

Bipolar disorder affects approximately 5.7 million adult Americans, or about 2.6% of the U.S. population age 18 years and old every year. The median age of onset for bipolar disorder is 25 years although the illness can start in early childhood or as late as the 40's and 50's. The overall frequency of the A allele is about 23% and it is the major allele in 51% of African populations.The A allele is also known as disease associated allele. The disease-associated allele is present in only 12% of individuals in Asia and in 15% of individuals in Europe (Drange OK,2019). There are many reasons for the occurrence of bipolar disorder such as genetics, poor mental health and environmental factors. Many studies has been done regarding bipolar disorder using GWAS. Genome Wide Association Studies (GWAS) is a method in research which identifies genomic variants which are associated with a disease or a certain characteristic. This analysis involves surveying the genomes of people, identifying the most repeated genes related to that particular disease. GWAS is used to examine only the susceptible loci. But it does not derive the connection between the disease and the SNPs, so gene ontology (GO) and Pathway analysis (PA) complements GWAS and extracts the significant biological features and functions which is used to the understand the interactions among genes. GWAS results are significantly affected by sample size. In this way PA is used to identify the potential susceptible locus and gene, thus complementing GWAS analysis. Thus, GWAS analysis is a powerful tool to explain the degree of contribution of each SNP to diseases. A GWAS study contains information about the associated SNPs of the disease and its p values respectively.

1.1GENES ASSOCIATED WITH BIPOLAR DISORDER

As finding a gene is the aim of this study the important genes associated with bipolar disorder will be discussed. As bipolar disorder is a psychiatric disorder the genes which are expressed in brain will be discussed. There are many genes expressed in the brain. Genes mentioned here have a significant relevance related to bipolar disorder according to literature published and human brain section in human protein atlas.

Tetratricopeptide repeat and ankyrin repeat containing 1 (TRANK 1) is a risk gene of bipolar disorder (BD). A study helps to reveal that gut microbiota may participate in the BD pathogenesis by interacting with a robust BD risk gene by the investigation of TRANK 1 expression on the morphology of neurons via transfection with plasmid Cytomegalovirus DNA (pcDNA3.1(+)) vector encoding human TRANK1(Lai Jianb0 *et al...*,, 2021) . Overexpression of TRANK1 is possibly associated with brain dysfunction by hampering the growth of dendritic spines and disrupting synaptic functions. TRANK1 encodes a protein of unknown function expressed in brain and other tissues(Jiang *et al...*,, 2019). TRANK1 is part of cluster 40 Granulosa cell. Decreased expression of TRANK1 perturbed expression of many genes involved in neural development and differentiation. Valproic acid (VPA) had the greatest effects on TRANK1 expression in iPSC, NPC, and astrocytes.TRANK1 gene is highly expressed in the cerebral cortex of the brain(Zhong.,2020).

The CACNA1C gene provides instructions for making one of several calcium channels. Calcium channels, which transport positively charged calcium atoms (calcium ions) into cells. Calcium channels play a key role in a cell's ability to generate electrical signals (Stanawaska *et al.* 2016). Calcium ions are important for many cellular functions, including regulating the electrical activity of cells, cell-to-cell communication, the tensing of muscle fibres and the regulation of certain genes, particularly those involved in the development of the brain and bones

before birth. A GWAS study profiled the DNA methylation landscape of five CGIs in CACNA1C in blood-derived DNA and found CGI 3 to be significantly hypermethylated in BD subjects compared with controls and methylation was influenced by gender as well as by nearby genotypes (Stanawaska *et al...*, 2016). The study concludes that CACNA1C DNA methylation may play a role in BD as the regulatory effect of risk alleles in intron 3 is accompanied by an upwards shift in DNA methylation of the intron 3 CGI. It is highly expressed in the amygdala in the brain(Karlsson.,2019).

Ankyrins are peripheral membrane proteins thought to interconnect integral proteins with the spectrin-based membrane skeleton. The ANK3 is located mainly at the nodes of Ranvier and the axon initial segment (AIS), 2 sub compartments of neurons responsible for the generation of action potentials . It has been shown to associate with the voltage-dependent sodium channel. ANK3 has been strongly implicated as a risk gene for bipolar disorder (BD) by recent genome-wide association studies of patient populations. The loss-of-function allele rs41283526G has a strong protective effect against BD (Wirgenes *et al.*, 2014). It strongly suggests that elevated expression of the *ANK3* isoform incorporating exon ENS00001786716 may be a factor in the pathobiology of the disorder (Hughes *et al...*,, 2018). The analysis of human RNA data sets shows that the available data strongly support this hypothesis and previous GWAS findings are also consistent with it. It is highly expressed in the cerebellum of the brain. Neurocan is involved in the modulation of cell adhesion and consists of neurocan core protein and chondroitin sulphate (Wang *et al...*,, 2018). A study published in 2012 confirmed that NCAN genotype was strongly associated with manic symptoms but not with depressive symptoms. It is highly expressed in then cerebral cortex of the brain. NCAN is also known as CSPG3(Chondroitin Sulphate Proteoglycans.

Pleckstrin homology domain containing O1 is associated with extreme mood swings in bipolar disorder. It interacts with capping protein for the regulation of the actin cytoskeleton . It is located in the mitochondria. It is a tumour suppressor as it inhibits AKT mediated cell survival. It is highly expressed in the white matter of the brain. This particular gene is also involved in schizophrenia (Andreassen *et al...*,,2015). It is also a prognostic marker in renal cancer. Adducin 3 is a heteromeric protein and they are composed of subunits such as adducin alpha, beta and gamma. These subunits are responsible for the cell to cell contact in epithelial cells. They are originally found in erythrocytes. This gene is highly expressed in other diseases such as thyroid cancer, colorectal cancer, stomach cancer, carcinoid, cervical cancer and ovarian cancer apart from bipolar disorder. In humans it is highly expressed in the cerebral cortex of the brain. It is mainly located in the plasma membrane. It's splice variants are ADD1,ADD2 AND ADD3.Inter alpha trypsin inhibitor heavy chain 1 acta as an carrier of hyaluronan as a serum or as a binding protein to regulate the processes of localization, synthesis and degradation of hyaluronan .This is very important for the biological processes in the body. The heavy chains interact with the hyaluronan . This interaction plays an important role in ovulation and fertilization. This gene has also been a part of many inflammatory diseases. It is mainly located in the vesicles. The tissue expression cluster is mainly in liver(Zhong.,2020).

Glutamate ionotropic receptor NMDA type subunit 2A is a complex which functions hetero tetrameric. These complexes are sensitive to magnesium. Binding of the neurotransmitter glutamate to the epsilon subunit, glycine binding to the zeta subunit, plus membrane depolarization leads to channel activation and eliminate channel inhibition by Mg(2+). GRIN1 and GRIN2A have lower sensitivity to glutamate. They contribute to the slow phase

of excitatory postsynaptic current, long-term synaptic potentiation, and learning. NMDA receptors are both ligand-gated and voltage-dependent, and are involved in long-term potentiation, an activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning. These receptors are permeable to calcium ions, and activation results in a calcium influx into post-synaptic cells, which results in the activation of several signalling cascades. Disrupted GRIN2A gene is associated with focal epilepsy and speech disorder with or without cognitive disability. Multiple transcript variants are due to alternative splicing(Karlsson.,2019).

The role of the N-methyl-D-aspartate receptor 2A subunit (GRIN2A) gene is associated with bipolar disorder by the region of 16p13.3 according to the two reasons which are a functional and polymorphic (GT)n repeat in the 5' regulatory region of the gene, with longer alleles showing lower transcriptional activity and an over representation in schizophrenia and a suggestion of genetic overlap between affective disorder and schizophrenia (Itokawa *et al.*, 2003). It is mainly expressed in the cerebral cortex of the brain. It's tissue expression mainly is in neuron signalling. It's splice variants are GRIN2A-201, GRIN2A-202, GRIN2A-205 GRIN2A -206, GRIN2A-212 GRIN2A-213, GRIN2A,-215, GRIN2A-216 and GRIN2A-218.Star related lipid transfer domain containing 9 is a microtubule motor protein. It is a protein which is necessary for spindle pole assembly during mitosis. It is also required to stabilize the pericentriolar material. It is expressed mainly in the white matter of the brain. It is an intracellular protein which is in liver, kidney, pancreas, lung , heart muscle, skeletal muscle and skin as well. The cell line expression cluster mainly is found in the nervous system. It's splice variants are STARD9-201 and STAR9-207. This gene is also identified by the eQTL method and its loci is 13q14.11(Zhong.,2020). **VAC14** component of PIKFYVE complex regulates the synthesis and turnover of phosphatidylinositol 3, 5- biphosphate. It activates PIKfyve kinase activity and it is also responsible for the biogenesis of endosome carrier vesicles (ECV) and multivesicular bodies (MVB) transport intermediates from early endosomes. It is highly expressed in the cerebral cortex of the brain. The tissue cell type of VAC14 gene is macrophages and it is a prognostic marker in renal and cervical cancer. It is mainly located in the vesicles. Its splice variants are VAC14-201, VAC14-202,VAC14-207 and VAC14-209

Microtubule affinity regulating kinase 2(MARK2) gene localizes and activates histone deacetylases by mediating phosphorylation of HDAC7 by the interaction between HDAC7 and 14-3-3 and export from the nucleus. It also acts a positive regulator of the Wnt signalling pathway. It's also essential for the development of membrane domains of polar epithelial cells. It's tissue expression cluster is mainly found in squamous epithelial cells. It is highly expressed in the white matter of the brain. It's tissue cell type is keratinocytes. It' a prognostic marker in liver and endometrial cancer. The MARK2 gene is located in the plasma membrane. It is present in the cytosol compartments and it's pathway is present in sub-system associated compartments.MARK2 and VAC14 genes are associated with bipolar disorder. These genes have a role in neurodevelopmental and neurodegenerative processes. The SNP of MARK2 gene is rs10792421 . The MARK2 gene encodes the microtubule affinity regulating kinase 2 (MARK2). The kinase is involved in a diversity of neuronal cellular processes, including neuronal migration, and tau phosphorylation. Bipolar disorder is also considered a neurodevelopmental disorder partly because of cortical cell migration abnormalities. The intronic variant within VAC14 rs11649476 is related to bipolar disorder.

(Karlsson.,2019).Many GWAS studies has been performed related to bipolar disorder using the gene expression in the brain. In this study, using GWAS, RRP11-152P17.2 gene is hypothesized to be related to bipolar disorder using it.s expression level in the brain.

REVIEW OF LITERATURE

The study conducted a GWAS on a large sample of individuals of European descent, including both cases and controls, and identified 30 loci that were genome-wide significant, 20 of which were newly identified. These significant loci contained genes responsible for ion channels, neurotransmitter transporters, and synaptic components.Furthermore, pathway analysis revealed nine gene sets that were significantly enriched, including the regulation of insulin secretion and endocannabinoid signaling. The study also found a strong genetic correlation between bipolar I disorder and schizophrenia, while bipolar II disorder was correlated with major depressive disorder.In summary, this study provides important insights into the genetic basis of these disorders and their shared genetic risk factors, which may lead to the development of new treatments and therapies in the future.(Stahl *et al*...,, 2019).

Recent GWAS studies on bipolar disorder (BD) have identified several significant associations between disease status and common genetic variants. The first large collaborative BD GWAS by the Psychiatric Genomics Consortium Bipolar Disorder Working Group identified four genome-wide significant loci, and subsequent meta-analyses identified an additional five loci.The proportion of variance in liability attributable to common variants (SNP-heritability) indicates that around 30% of the heritability for BD is due to common genetic variants. However, only a small fraction of this heritability has been explained by associated loci so far.In this study, the PGC Bipolar Disorder Working Group conducted a second GWAS with a larger sample size, comprising 20,352 cases and 31,358 controls of European descent, and followed up on top findings in an independent sample of 9,412 cases and 137,760 controls. While some of the findings reinforce specific hypotheses regarding BD neurobiology, the majority of the findings suggest new biological insights.Overall, this study provides valuable insights into the genetic basis of BD and may inform the development of new treatments and therapies in the future by identifying potential targets for intervention.(Stahl *et al*...,, 2019).

A large bipolar disorder (BD) GWAS study identified 30 genome-wide significant loci, including 20 that were novel. Previous BD GWAS have reported a total of 20 loci significantly associated with BD9–23 ; twelve of these previously reported loci were not genome-wide significant in our GWAS meta analysis, but all had PGWAS ≤ 1.3×10−5. Recent GWAS of BD and SCZ 52, which included our discovery GWAS data jointly analyzed with published SCZ data 31 (without overlapping control subjects), highlighted similarities and differences in BD and SCZ in terms of known associated SNPs and PRS-subphenotype associations; here the power was maximized to identify BD associations. Phenotypic variance explained by polygenic risk scores (PRS) based on thr BD GWAS data is ~8% (observed scale; 4% on the liability scale 53), an increase from 2.8% (1.2% on the liability scale) in our previous study 9 . The results of the BD subtype PRS analyses support the nosological distinction between BD1 and BD2, but also highlight the importance of psychosis beyond DSM subtypes, corroborating and expanding

evidence from previous clinical 54 and genetic studies 52,55,56. The DEPR vs. BD PRS analyses provide further support for the distinction between BD1 and BD2, independent of the presence of psychosis(Stahl *et al.*, 2019).

Alzheimer's disease (AD) and bipolar disorder (BIP) are complex traits influenced by numerous common genetic variants, most of which remain to be detected. Clinical and epidemiological evidence suggest that AD and BIP are related. A study applied statistical methods based on the conditional false discovery rate (FDR) framework to detect genetic overlap between AD and BIP and utilized this overlap to increase the power to identify common genetic variants associated with either or both traits. Genome wide association studies data from the International Genomics of Alzheimer's Project part 1 (17,008 AD cases and 37,154 controls) and the Psychiatric Genetic Consortium Bipolar Disorder Working Group (20,352 BIP cases and 31,358 controls). Conditional QQ-plots was used to assess overlap in common genetic variants between AD and BIP. The genetic overlap was used to re-rank test-statistics for AD and BIP and improve detection of genetic variants using the conditional FDR framework. Conditional QQ-plots demonstrated a polygenic overlap between AD and BIP. Using conditional FDR, one novel genomic locus was associated with AD, and nine novel loci associated with BIP. Two novel loci were identified to be commonly associated with AD and BIP implicating the MARK2 gene with the lead SNP of rs10792421, conjunctional FDR = 0.030, same direction of effect and the VAC14 gene with the lead SNP of rs11649476, conjunctional FDR = 0.022, opposite direction of effect. The study was concluded polygenic by the identification of overlap between AD and BIP and novel loci for each trait and two jointly associated loci (Drange OK,2019).

Alzheimer's disease is a neurodegenerative disorder (Jack *et al.*, 2013) usually presenting in late adult life (Koedam *et al*., 2010), while BIP is considered a neurodevelopmental disorder (Sanches *et al*...,., 2008; O'Shea and McInnis, 2016) with average age at onset in early adult life (Baldessarini *et al.*, 2010). Yet, epidemiological, pathophysiological, and clinical data suggest that AD and BIP could be related. A recent meta-analysis reports an odds ratio of 2.4 (95% CI 1.4–4.1) for dementia of all causes among patients with BIP (Diniz *et al*...,., 2017). The risk of dementia is higher among patients with BIP compared to patients with arthritis, diabetes, and schizophrenia (Kessing *et al.*, 1999; Kessing and Nilsson, 2003). Among patients with BIP, treatment with lithium is associated with a reduced risk of dementia (Kessing *et al.*, 2010; Gerhard *et al.*, 2015) and AD (Nunes *et al.*, 2007) in most, but not all (Cheng *et al*...,., 2017), observational studies. Among patients with AD or mild cognitive impairment, a meta analysis of randomized controlled studies found that lithium decreased cognitive decline (Matsunaga *et al*,, 2015). Shared pathophysiological processes between AD and BIP are reported in the kynurenine pathway (Miller *et al*...,., 2006; Myint *et al*...,., 2007; Rahman *et al*...,., 2009; Gulaj *et al*...,., 2010; Maddison and Giorgini, 2015; Savitz *et al*...,., 2015). There is also evidence of inflammatory processes in both conditions (Goldstein *et al*...,., 2009; Antonio *et al*...,., 2015; Heneka *et al*...,., 2015). Further, euthymic patients with BIP have impairments of episodic memory (Torres *et al*...,., 2007) and executive dysfunction (Torres *et al*...,., 2007; Martino *et al*...,., 2015), which are also core symptoms of AD (Gold and Budson, 2008; Godefroy *et al*...,., 2016). Despite several lines of evidence suggesting a relation between AD and BIP, it is not established if the conditions have a shared genetic basis. AD and BIP are in most cases complex traits, i.e., they are influenced by several genetic and environmental factors. Twin studies estimate the heritability of AD and BIP to60% or higher (McGuffin *et al*...,., 2003; Kieseppä and Partonen, 2004; Gatz *et al*...,., 2006; Lichtenstein *et al*...,., 2009). Genome wide association studies (GWASs)

are the gold standard for hypothesis-free assessment of associations between complex traits and common genetic variants (Corvin *et al.*..,., 2010). The common variants refer to single nucleotide polymorphisms (SNPs) with minor allele frequencies > 1–5%. The power of a GWAS is a function of study sample size and the genetic architecture of the trait (i.e., the narrow-sense heritability, the number of causal variants, their effect sizes, and population frequencies) (Schork *et al.*..,., 2016; Frei *et al.*..,., 2018). AD and BIP are considered highly polygenic (Purcell *et al.*..,., 2009; Escott-Price *et al.*..,., 2015), and ∼1/3 of their heritability can be explained by SNPs with tiny effect sizes that are not individually detectable given the power of current GWASs (Lee *et al.*..,., 2011, 2013; Ridge *et al.*..,., 2013, 2016). With the current sample sizes, however, the power of GWASs can be boosted by leveraging polygenic overlap between complex traits (Andreassen *et al.*..,., 2013a,b, 2015). Shared genetic influences are common among complex traits (Visscher *et al.*..,., 2017). Statistical methods based on the conditional FDR framework can detect polygenic overlap between complex traits and utilize this polygenic overlap to increase the power to identify common genetic variants associated with each trait and jointly with two or more traits (Andreassen *et al.*..,., 2013, 2015). We aimed to use these methods to identify the shared genetic basis between AD and BIP(Drange OK *et al.*..,., 2019).

Besides genome-wide association studies, a variety of other genetic analyses such as polygenic risk scores, whole-exome sequencing and whole-genome sequencing have been conducted for investigating the involvement of common, rare and very rare types of DNA sequence variants in bipolar disorder. Similarly, non-invasive neuroimaging methods can be used to quantify changes in brain structure and function in patients with bipolar disorder. The aim of this study was to provide a comprehensive assessment of genetic findings associated with bipolar disorder, based on the evaluation of different genomic approaches and neuroimaging studies. A PubMed search of all relevant literatures was conducted from the beginning to the present, by querying related search strings. ANK3, CACNA1C, SYNE1, ODZ4 and TRANK1 are five genes that have been replicated as key gene candidates in bipolar disorder pathophysiology, through the investigated studies. Bipolar disorder polygenic risk scores are associated with other psychiatric phenotypes. The ENIGMA-BD studies show a replicable pattern of lower cortical thickness, altered white matter integrity and smaller subcortical volumes in bipolar disorder. The low amount of explained phenotypic variance highlights the need for further large-scale investigations, especially among non-European populations, to achieve a more complete understanding of the genetic architecture of bipolar disorder and the missing heritability. Combining neuroimaging data with genetic data in large-scale studies might help researchers acquire a better knowledge of the engaged brain regions in bipolar disorder(Oraki *et al.*...,2022).

Psychiatric genetics, formed in the 20th century, is a branch of behavioural genetics that investigates the role of genetics in the onset and progression of mental illnesses. Attempts to use genomic data for prediction and risk stratification have been classically hampered by the highly polygenic and pleiotropic nature of psychiatric genetics. Current models suggest that bipolar disorder is a heterogeneous and multifactorial disease, which probably involves several pathogenic pathways orchestrated by numerous common and rare genetic factors, environmental factors and epigenetic influences. Over the past several decades, many genetic studies have been performed to uncover the genetic underpinnings of bipolar disorder. For complex disorders like bipolar disorder, association studies are expected to be more informative than linkage studies because of their higher statistical power to capture modest

genetic effects. However, initial candidate gene approaches suffered a challenging lack of replicability of the results that raised questions regarding the actual utility of association studies in psychiatric genetics. Genome-wide association studies (GWASs), which were introduced as a powerful alternative to linkage and a hypothesis-free extension of candidate gene studies, have emerged as a revolutionary approach in research on complex genetic disorders. GWASs aim to reveal genotype-phenotype associations by testing the association between millions of common genetic variants distributed across the human genome with the phenotype of interest. GWASs have provided compelling evidence of the ability to unravel the genetic underpinnings of complex traits. One important lesson from GWASs is that the cumulative number of loci to be discovered in GWASs is growing in line with the increasing sample size(Oraki *et al*...,,2022).

In genetic association studies, which investigate many genetic variants to detect disease-associated ones, appropriate adjustment for multiple comparison is needed for distinguishing true association and avoiding a flood of false-positive cases. The effectiveness of genetic association studies to discover causative genes of complex disorders requires a sample size with appropriate statistical power, which will be influenced significantly by disease prevalence, disease allele frequency, linkage disequilibrium, inheritance models and effect size of the genetic variants. The smallest sample size required to generate appropriate statistical power (e.g. 80% power) is known as an effective sample size. In large-scale association studies, the statistical power of 80% is widely used to eliminate false-negative associations and establish the most cost-effective sample size.The evidence collected over the past years indicates that bipolar disorder has an extremely polygenic architecture, with many genetic variants shaping the individual genetic vulnerability for this disorder. Polygenic risk scores (PRS), also referred to as polygenic scores, provide an estimate of this genetic vulnerability and represent a genetic tool with the potential to identify people who are at risk for bipolar disorder or for a worse course of the disease(Oraki *et al*...,,2022).

A study uses data science technique to identify the important Single Nucleotide Polymorphisms (SNPs) significantly affecting the classifications of BD-I and BD-II, and develops a set of complementary diagnostic classifiers to enhance the diagnostic process. Screening assessments and SNP genotypes of 316 Han Chinese were performed with the Affymetrix Axiom Genome-Wide TWB Array Plate. The results show that the classifier constructed by 23 SNPs reached the area under curve of ROC (AUC) level of 0.939, while the classifier constructed by 42 SNPs reached the AUC level of 0.9574, which is a mere addition of 1.84%. The accuracy rate of classification increased by 3.46%. This study also uses Gene Ontology (GO) and Pathway to conduct a functional analysis and identifies calcium ion binding, GABA-A receptor activity, Rap1 signaling pathway, ECM proteoglycans, IL12-mediated signaling events, Nicotine addiction), and PI3K-Akt signaling pathway(Chia *et al*...,,2020)

In literature, there are several techniques developed for variable selection; however, most of methods on GWAS dataset tend to misunderstand the causal SNPs that are marginally uncorrelated with disease or have high false discovery rates (FDRs). The GWASelect regression-based method has the ability to capture both marginally correlated and marginally uncorrelated causal SNPs and has low FDR [14]. Apart from GWAS data can be used classify different disease in clinically, the real fMRI (functional magnetic resonance imaging) data set can also be applied for categorizing the participants into three classes including healthy controls, BD-II, and schizophrenia

patients [15]. In addition, next-generation sequencing (NGS) data also has been widely used for DNA variant discovery [16]. An integrated simulation system (IntSIM) was developed to conduct effective simulations of NGS data with many realistic features. The experiments included common types of germline (SNP and CNV) and somatic variants (SNV and CNA) from NGS genomes. IntSIM adopt hidden Markov models(HMM) trained from real genomes to predict the states of SNP, CNV (copy number variations), and CNA (copy. number alterations); maintaining the inherent correlations among variant markers across genome.

A novel data science framework to analyze the genome-wide association study (GWAS) data including data preprocessing, univariate analysis, feature selection, evaluation and comparison, described as follows. The single-marker analysis is used to identify the SNPs that showed the main effects significantly affecting BD. Four steps were used to ensure SNP quality control: (1) removing SNPs with more than 5% genotyping missing rate; (2) removing SNPs that failed to satisfy the Hardy-Weinberg equilibrium (HWE), with the threshold being 10-5 ; (3) removing SNPs with minor allele frequency (MAF) less than 5%; and (4) linkage disequilibrium (LD)-based SNP pruning generates a subset of SNPs in approximate linkage equilibrium with each other, with a threshold r2>0.2 [17]. Linkage disequilibrium (LD) exists because of the shared ancestry of contemporary chromosomes. Strong linkage between loci on the same chromosome could result in high levels of LD [18]. PLINK's indep-pairwise was suggested with parameters (window size, step, r 2 threshold) = (50, 5, 0.2) command to prune dataset for LD. The advantage of LD pruning can retain a larger proportion of potentially causal variants. This also particularly benefits GWAS studies with low computing power and reducing multiple testings [19]. Furthermore, LD pruning creates an independent set supporting the data mining afterward. The proposed four-step quality control can remove a large amount of uncorrelated SNPs; particular, for the clinical application, it is capable to classify whether a patient is BD-I or BD-II with only a few numbers of SNPs(Chia *et al.*, 2020)

Using data science, many studies has been performed related to bipolar disorder. Specifically using classifiers, many studies have been performed to distinguish between the genes of bipolar disorder and schizophrenia as they are closely related by symptoms. Research related to other psychiatric disorders such as Major Depressive Disorder(MDD), Autism and Attention-Deficit Hyperactivity disorder(ADHD).

AIM AND SCOPE OF PRESENT INVESTIGATION

The aim of this study is to find a gene related to bipolar disorder which is not reported yet. Using Genome Wide Association Studies(GWAS) data of bipolar disorder, the aim of this study is to find possible target/ candidate gene related to bipolar disorder. It's objectives include

- Downloading GWAS dataset and VCF files
- Taking the SNPs from the dataset only responsible for bipolar disorder
- Performing GSEA to find the potential target/candidate gene
- Finding the gene expression of the hypothesized gene

The scope of this study is it may add a gene to the list of existing genes related to bipolar disorder. As this study uses GWAS to find a potential target /candidate gene the results can be accurate and has a reliable research potential. As GWAS encompasses statistically significant genes, the resulting output has a high probability of being a significant gene related to bipolar disorder. Here the gene RRP11-152P17.2 is hypothesized to be related to bipolar disorder. This study can help to identify a possible gene related to bipolar disorder by adding a gene to the existing list of genes related to bipolar disorder.

MATERIALS AND METHODS

In this study, a thorough investigation was conducted on bipolar disorder, a mental health condition characterized by extreme mood swings. To gather relevant information, several sources were utilized, including research papers from PubMed Central and databases such as Human Brain Atlas and Gene Ontology (GO).PubMed Central is a full-text repository that houses published research papers in its database. By searching PubMed, abstracts of publications can be accessed and searched. However, the same publication in PubMed Central contains the full-text article, and the full-text is searchable.

The Human Brain Atlas was used to identify genes associated with bipolar disorder and obtain information on their function, role in the disorder, co-expression, location, and splice variants. The study employed various tools such as rSNPbase, FUMA GWAS, enrichr, and Webgestalt for Gene Set Enrichment Analysis (GSEA) to analyze the expression of these genes.GSEA is a powerful method for interpreting gene expression data. It evaluates the cumulative changes in the expression of groups of genes defined based on prior biological knowledge. It first ranks all genes in a data set, then calculates an enrichment score for each gene set, which reflects how often members of that gene set occur at the top or bottom of the ranked data set. This helps to determine if there are statistically significant, cumulative changes in gene expression that are correlated with a phenotype, which in this case is bipolar disorder.

The study also utilized the Gene Ontology (GO) to categorize our understanding of the biological domain into three aspects: molecular function, biological process, and cellular component. Molecular function terms describe activities rather than entities and include activities performed by individual gene products or molecular complexes. Cellular component classes refer to cellular anatomy, while biological process terms describe larger processes accomplished by multiple molecular activities.However, it is important to note that the GO does not aim to represent pathway dynamics or dependencies. Therefore, other methods or tools may be needed to analyze such aspects. Overall, the use of these resources and tools can provide valuable insights into the molecular mechanisms underlying bipolar disorder and help guide future research and treatment strategies.

4.1 DOWNLOADING DATASET AND VCF FILE FORMAT

A dataset is a collection of data. It usually contains rows and columns. It contains values and numbers and information related to a particular subject. Variant Call Format is a file format which is the output of a bioinformatics pipeline. The DNA sample sequenced into raw sequence. The sequence is aligned into aligned reads. The reads are called as variant calls. This file is called variant call format. It has three main sections namely meta

section, header line and data lines. The meta section contains the information about the variant calling software or the reference genome for determining variants and the sequencing conducted . It also includes the fields such as INFO which is the site level and the FORMAT which is the sample level . This section starts with #. It includes the following fields.

#CHROM POS ID REF ALT QUAL FILTER INFO

This section also includes a genotype data under the FORMAT column which is represented with the help of unique sample names. Each data line is represented as a position in the genome. Under the header line data lines are present and they are separated by tabs and ends with a newline. A dot represents missing values in a data line.

#CHROM - It is the chromosome identifier.

POS – It is the reference position which is sorted in ascending order by chromosome.

ID – It is the unique identifiers which are separated by semicolons and no whitespaces are allowed.

REF – It is the reference base and insertions are represented by a dot.

ALT- It is the Alternate base which are separated by comma and deletions are represented by a dot.

QUAL – It is the quality score that is based on a log scale.

FILTER - It indicates which filters have failed or passed and they are separated by semicolons.

FORMAT – It is the sample level field name which are separated by semicolons.

<SAMPLE DATA> - It is the sample level field data according to the FORMAT field and are separated by semicolons .

4.2 GENE SET ENRICHMENT ANALYSIS (GSEA) OF GWAS GENES AND SNPs

Gene set enrichment analysis (GSEA) is a powerful tool to associate a disease phenotype to a group of genes/proteins. GSEA attributes a specific weight to each gene/protein in the input list that depends on a metric of choice, which is usually represented by quantitative expression data. However, expression data are not always available. GSEA helps researchers to perform pathway enrichment using gene expression data sets. It is particularly suitable when expression data are available for all the genes under investigation. GSEA differs from differential gene expression analysis in the sense that it might identify genes which are part of a differentially expressed set but which might not be identified as significantly differentially expressed alone. Two datasets were used for gene set enrichment analysis. The first was a GWAS dataset in a VCF file format from figshare website (https://figshare.com). The second dataset was collected from dbBIP(Shun shai,2022) which is database of bipolar disorder and it's genetic research. It had a GWAS dataset from Psychiatric Genomics Consortium (PGC).

The Psychiatric Genomics Consortium (PGC) is an international consortium of scientists dedicated to conducting meta- and mega-analyses of genomic-wide genetic data, with a focus on psychiatric disorders. It is the largest psychiatric consortium ever created including over 800 researchers from 38 countries as of 2019. Its goal is to generate information about the genetics of psychiatric conditions that is, "genetic findings whose biological implications can be used to improve diagnosis, develop rational therapeutics, and craft mechanistic approaches to primary prevention". The consortium makes the main findings from its research freely available for use by other researchers.[3] Research from the PGC has shed light on the genetic architecture of psychiatric disorders generally, as well as demonstrating the viability of the genome-wide association approach for specific disorders such as schizophrenia and bipolar disorder. The consortium has also identified 108 genetic loci that are consistently associated with schizophrenia. In addition, its findings have pointed to significant pleiotropy across psychiatric disorders, with many common alleles influencing the risk of multiple such disorders.]The p values from both datasets were taken into consideration to look for which genes are highly responsible for the occurrence of the disease. The analysis of GWAS data from two datasets were performed by taking 137 SNPs that had p values less than 0.05. They were annotated using rSNPBase 3.1 database (Wang L,2018((http://rsnp3 .psych.ac.cn/). rSNPbase which provides human SNP-related regulatory elements, element-gene pairs and SNP-based regulatory networks. For those SNPs 44 target genes were found.Gene Set Enrichment analysis was done using FUMA GWAS, webgestalt and Enrichr.

Functional mapping and annotation for Genome wide association studies (FUMA GWAS) (https://fuma.ctglab.nl/) is a platform that can be used to annotate, prioritize, visualise and interpret GWAS results. The SNP2GENE function takes GWAS summary statistics as an input, and provides extensive functional annotation for all SNPs in genomic areas identified by lead SNPs. The GENE2FUNC function takes a list of gene IDs (as identified by SNP2GENE or as provided manually) and annotates genes in biological context. FUMA GWAS identified five unknown target genes from those 44 target genes. FUMA, which functionally annotates GWAS findings and prioritizes the most likely causal SNPs and genes using information from 18 biological data repositories and tools. Gene prioritization is based on a combination of positional mapping, expression quantitative trait loci (eQTL) mapping and chromatin interaction mapping. Results are visualized to facilitate quick insight into the implicated molecular functions. FUMA is available as an online tool at (Watenbe,2017)(http://fuma.ctglab.nl) where users can customize settings to for example only use exonic SNPs for annotation, or only use SNPs that are eQTLs in specific tissues for the annotation based on expression data. As input, FUMA requires GWAS summary statistics and outputs include multiple tables and figures containing extensive information on, e.g., functionality of SNPs in genomic risk loci, including protein-altering consequences, gene-expression influences, open-chromatin states as well as three-dimensional (3D) chromatin interactions. The online tool includes interactive figures that can

be used to explore associations in more depth and aids, e.g., in identifying multiple lines of evidence pointing to the same prioritized gene, or in connecting hits in several genes via biological pathways.

WebGestalt(Web based gene analysis toolkit)(Zhang *et al*...,.,2005) (http://www.webgestalt.org/) is a popular tool for the interpretation of gene lists derived from large scale -omics studies. In the 2019 update, WebGestalt supports 12 organisms, 342 gene identifiers and 155 175 functional categories, as well as user-uploaded functional databases. WebGestalt (WEB-based GEne SeT AnaLysis Toolkit) has become one of the popular software tools in this field since its publication in 2005. For the last 7 years, WebGestalt data holdings have grown substantially to satisfy the requirements of users from different research areas. The current version of WebGestalt supports 8 organisms and 201 gene identifiers from various databases and different technology platforms, making it directly available to the fast growing omics community.

Meanwhile, by integrating functional categories derived from centrally and publicly curated databases as well as computational analyses, WebGestalt has significantly increased the coverage of functional categories in various biological contexts including Gene Ontology, pathway, network module, gene-phenotype association, gene-disease association, gene-drug association and chromosomal location, leading to a total of 78 612 functional categories. Finally, new interactive features, such as pathway map, hierarchical network visualization and phenotype ontology visualization have been added to WebGestalt to help users better understand the enrichment results. WebGestalt can be freely accessed through http://www.webgestalt.org or http://bioinfo.vanderbilt.edu/webgestalt/.Further analysis was done by Enrichr to find more information about the target genes.

Enrichr(Chen *et al*...,, 2013)(Enrichr maayanlab.cloud) currently contains a large collection of diverse gene set libraries available for analysis and download. In total, Enrichr currently contains 180 184 annotated gene sets from 102 gene set libraries. Enrichr is a comprehensive resource for curated gene sets and a search engine that accumulates biological knowledge for further biological discoveries. Enrichr (Chen *et al*...,, 2013; Kuleshov *et al*...,, 2016) is a gene set search engine that enables the querying of hundreds of thousands of annotated gene sets. Enrichr uniquely integrates knowledge from many high-profile projects to provide synthesized information about mammalian genes and gene sets. The platform provides various methods to compute gene set enrichment, and the results are visualized in several interactive ways.It includes analyzing lists of differentially expressed genes from transcriptomics, proteomics and phosphoproteomics, GWAS studies, or other experimental studies, searching Enrichr by a single gene or key search term, preparing raw or processed RNA-seq data through BioJupies in preparation for Enrichr analysis, analyzing gene sets for model organisms using modEnrichr,using Enrichr in Geneshot,using Enrichr in ARCHS4,using the enrichment analysis visualization Appyter to visualize Enrichr results

RRP11-152P17.2 is one of the 44 target genes whose expression was identified in brain by GTEX (https://gtexportal.org/home/). GTEX was used to find the expression level of RRP11-152P17.2 in the brain.The

Genotype-Tissue Expression (GTEx) aims to provide a resource with which to study human gene expression and regulation and its relationship to genetic variation. It collects and analyses multiple human tissues from donors who are also densely genotyped, to assess genetic variation within their genomes. By analysing global RNA expression within individual tissues and treating the expression levels of genes as quantitative traits, variations in gene expression that are highly correlated with genetic variation can be identified as expression quantitative trait loci, or eQTLs. The GTEx project of the NIH Common Fund aims to establish a resource database and associated tissue bank in which to study the relationship between genetic variation and gene expression and other molecular phenotypes in multiple reference tissues.

The GTEx project began with a 2.5-year pilot phase to test the feasibility of establishing a rapid autopsy program that would yield high-quality nucleic acids and robust gene expression measurements. Having met milestones of donor enrollment, RNA quality and eQTL findings, the project is scaling up to include approximately 900 post-mortem donors by the end of 2015. The power to detect eQTLs is dependent on multiple factors that are difficult to quantify precisely, but power estimates over a range of effect sizes and allele frequencies are described. Most associated variants are not correlated with protein-coding changes, suggesting that polymorphisms in regulatory regions probably contribute to many disease phenotypes. The Genotype-Tissue Expression (GTEx) project establishes a resource database and associated tissue bank for the scientific community to study the relationship between genetic variation and gene expression in human tissues.

RESULTS AND DISCUSSIONS

TABLE 5.1 RESULTS FROM rSNPbase DESCRIBING THE DOWNSTREAM TARGET GENES OF THE SNPs

SNPS	DOWNSTREAM TARGET GENES
rs9834970	MOBP,CTDSPL,ENTPD3-AS1
rs12226877	FEN1
rs4447398	STARD9,RTF1
rs112114764	HDAC5,RYBP,C17orf53,ATXN7L3
rs7014597	BAALC
rs1786334	RGS22,UBR5
rs77519446	ZNF706

s1497632	ZFPM2-AS1,CSMD3,TMEM74
s6993873	ZFPM2-AS1,LRP12,RIMS2
s6981446	ZFPM2-AS1,OXR1
s16869498	KB-1980E6.3
s61638166	ZFPM2-AS1,KB-1980E6.3,RIMS2
s1434235	KB-1980E6.3
s6468965	ZFPM2-AS1,KB-1980E6.3,RIMS2
s16898906	ZNF706
s12541795	ADCY8,,CSMD3,ZFPM2,ARHGAP39,OXR1,
s2847729	RGS22,UBR5
s61852271	PARD3,NRP1,SVILP1,SVIL,PTCHD3P1
s12773169	PARD3,NRP1,SVILP1
s10827530	CUL2,PARD3,NRP1,SVILP1
s1969135	RN7SL63P
s111226391	PARD3,NRP1,SVILP1
s113933281	PARD3,NRP1,CCNY-AS1
s150331434	TGB1-DT ,SVIL,ARHGAP12
s11010879	PARD3
s140370967	PARD3,ITGB1-DT
s4934707	CUL2,PARD3,NRP1
s11598041	PPIAP31
s11010235	EPC1,CUL2,PARD3,SVILP1,CACNB2
s12240347	CUL2,PARD3,NRP1
s117857061	EPC1
s78009943	CUL2,PARD3,NRP1
s3006590	ZNF438
s608371	EPC1,CUL2,PARD3,SVILP1,CACNB2
s1418278	SVILP1,SVIL,PTCHD3P1
s79392837	CUL2,PARD3,NRP1
s76200555	PARD3,NRP1
s2998033	Y RNA
s682762	CUL2,PARD3,NRP1,SVILP1
s1832864	SVILP1,SVIL,PTCHD3P1

s1774241	SVILP1,SVIL,PTCHD3P1
s118186958	PARD3,NRP1
s2484854	CUL2,PARD3,NRP1,SVILP1

From rSNPbase results, 44 target genes were found for the 44snps which had p value less than 0.05 . The table encompasses the SNPs less than take from datasets. Every SNP has it's respective target genes. These target genes are used for gene set enrichment analysis(GSEA).

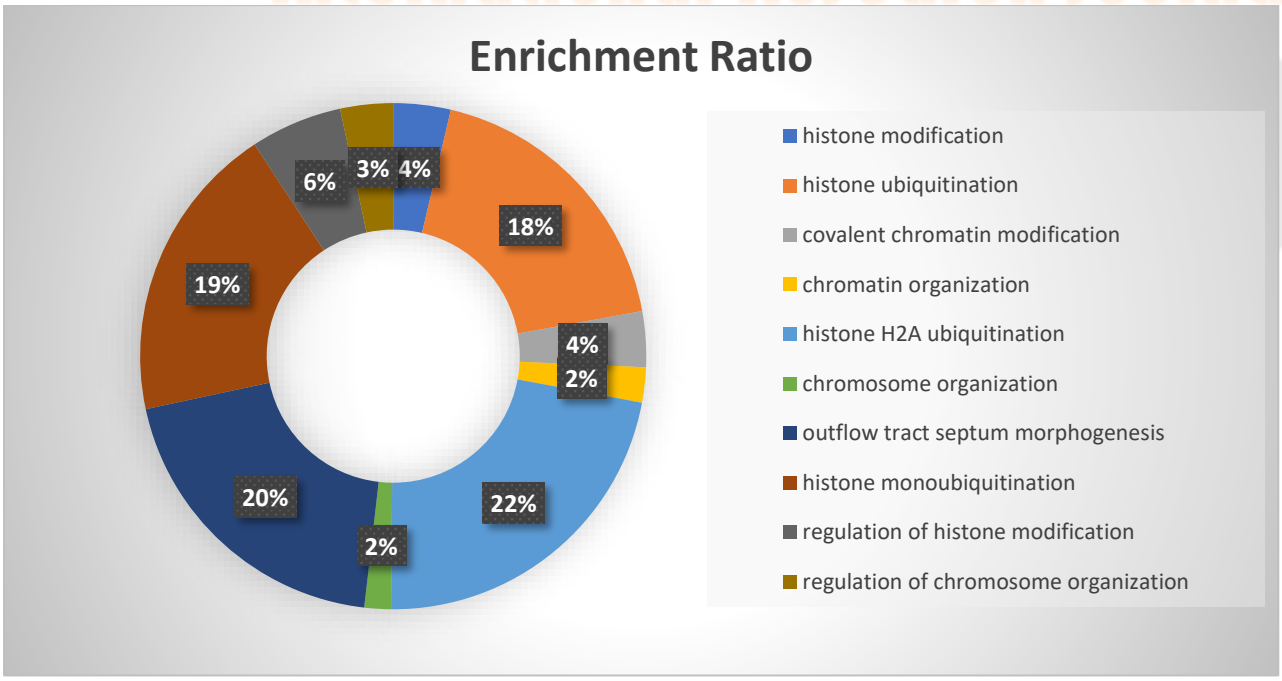

FIGURE 5.1 BIOLOGICAL PROCESSES RELATED TO THE TARGET GENES FROM WEBGESTALT

Those 44 target genes were used as input for FUMA GWAS, WEBGESTALT AND ENRICHR. Using FUMA GWAS and WEBGESTALT it was found that the most of the target genes were involved in Chromatin modification and histone ubiquitination. .As histones are the most abundant ubiquitinated proteins, their ubiquitination plays critical roles in many processes in the nucleus, including transcription, maintenance of chromatin structure, and DNA repair. Histone ubiquitination is largely related to neurodevelopmental disorders such as autism and major depressive disorder(Srivatsava *et al.*,2017).

Clusters of active non-coding parts of the linear genome marked by histone acetylation represent building blocks of 3D structure of the genome. The researchers showed that many chromatin domains with disordered acetylation in the brains of patients diagnosed with schizophrenia or bipolar disorder were commonly located deep inside the nerve cell nucleus in active compartments.Both messenger RNA and protein expression of HDACs 1, 3, and 5 have been shown to be altered in depression, bipolar disorder, and schizophrenia. A research was conducted at Icahn School of Medicine at Mount Sinai in New York specifically examining chromosomal organizations that are associated with the heritability of two major psychiatric conditions, namely schizophrenia and bipolar disorder.

TABLE 5.2 GENES ASSOCIATED WITH BIPOLAR DISORDER ACCORDING TO ENRICHR

Name	Associated genes
Corticobasal degeneration	MOBP
Schizophrenia or bipolar disorder	TRANK 1
Autism spectrum disorder, ADHD, bipolar disorder, MDD, and schizophrenia (combined)	CACNB2,ZFPM2
Bipolar disorder with mood-incongruent psychosis	TRANK1
Bipolar disorder	TRANK1,,ADCY8,HDAC5

The MOPB gene, also known as the MOBP gene, encodes a myelin-associated oligodendrocytic basic protein that is primarily expressed in the central nervous system.There have been several studies investigating the association between the MOPB gene and bipolar disorder, which is a mental health condition characterized by episodes of mania and depression.One study published in the journal Molecular Psychiatry in 2013 found a significant association between variants of the MOPB gene and bipolar disorder in a sample of European individuals(Hou.L *et al...*, 2013). More recently, a study published in the journal.Scientific Reports in 2019 found that a specific variant of the MOPB gene was associated with bipolar disorder in a sample of Han Chinese individuals. The MOPB gene codes for a protein that is involved in the formation and maintenance of myelin, which is the fatty substance that surrounds nerve fibers in the central nervous system and is important for proper neural function. Some studies have suggested that abnormalities in myelin may play a role in the

development and progression of bipolar disorder. In addition, the MOPB gene is located on chromosome 3, which has been linked to bipolar disorder in some genetic studies. Variants in the MOPB gene have been associated with alterations in brain structure and function, including changes in white matter integrity, which may be relevant to bipolar disorder(Russos *et al*...,,2012)

CACNB**2 (**Voltage-dependent L-type calcium channel subunit beta-2),Mutation in the CACNB2 gene are associated with Brugada syndrome, autism, attention deficit-hyperactivity disorder (ADHD), bipolar disorder, major depressive disorder, and schizophrenia. Calcium voltage-gated channel auxiliary subunit β2 is a protein that, in humans, is encoded by the *CACNB2* gene. The β2 subunit is an auxiliary protein of voltage-gated calcium channels, which is predominantly expressed in hippocampal pyramidal neurons. A single-nucleotide polymorphism at the *CACNB2* gene (rs11013860) has been reported in genome-wide association studies to be associated with bipolar disorder (BD). However, the neural effects of rs11013860 expression are unknown. Thus, the current study investigated the mechanisms of how the *CACNB2* gene influences hippocampal-cortical limbic circuits in patients with bipolar disorder (BD).A total of 202 subjects were studied [69 BD patients and 133 healthy controls (HC)]. Participants agreed to undergo resting-state functional magnetic resonance imaging (rs-fMRI) and have blood drawn for genetic testing. Participants were found to belong to either a CC group homozygous for the C-allele (17 BD, 41 HC), or an A-carrier group carrying the high risk A-allele (AA/CA genotypes; 52 BD, 92 HC). Brain activity was assessed using resting-state functional connectivity (rs-FC) analyses. A main effect of genotype showed that the rs-FC of the AA/CA group was elevated more than that of the CC-group between the hippocampus and the regions of right-inferior temporal, fusiform, and left-inferior occipital gyri. Additionally, a significant diagnosis × genotype interaction was noted between the hippocampus and right pars triangularis. Furthermore, in BD patients, the AA/CA group showed lower rs-FC when compared to that of the CC group. Additionally, individuals from HC within the AA/CA group showed higher rs-FC than that of the CC group. Finally, within C-allele-carrying groups, individuals with BD showed significantly increased rs-FC compared to that of HC. Our study demonstrates that BD patients with the *CACNB2* rs11013860 AA/CA genotype may exhibit altered hippocampal-cortical connectivity(Liu *et al*...,,2019).

Psychiatric disorders have a negative impact on society and human lives. Genetic factors are involved in the occurrence and development of psychiatric diseases. ZFPM2 has been identified as one of the most compelling risk genes associated with broad phenotypes related to psychosis. A systematic meta-analysis reviewed ZFPM2 variants in psychosis-related disorders, including schizophrenia, bipolar disorder, and attention-deficit hyperactivity disorder. We also summarized the association between other zinc finger protein genes (*ZNFs*) and psychiatric diseases. The meta-analysis included a total of six variants of *ZFPM2* and three variants of other *ZFPM2* and the effects of *ZFPM2* variants on neurocognition and neuroimaging phenotypes were reviewed.

The biological functions of these variants are also presented. It was verified that ZFPM2 was significantly related to psychiatric diseases, and the association between ZFPM2 rs1344706 and psychosis (schizophrenia and bipolar disorder) did not vary with disease or ethnicity. The main brain area regulated by ZFPM2 *rs1344706* was the dorsolateral prefrontal cortex. The effect *of* ZFPM2 *variants* on cognition did not display consistency with different diseases or methodologies. These findings suggest that *ZFPM2* might play an important role in common pathogenesis of psychiatric diseases, and its variants are likely involved in regulating the expression of psychosis-related genes, especially the dopamine pathway genes. Further research should focus on the molecular mechanisms by which ZFPM2 *va*riants act in psychiatric diseases and related phenotypes(Yan *et al.*, 2015).

During early postnatal life, *ADCY8* mRNA is expressed in hippocampal CA1 region, cortex, cerebellum, olfactory bulb, hypothalamus, amygdala and basal ganglia. In adulthood, *ADCY8* is found in olfactory bulb, cerebellum, hypothalamus, thalamus, hippocampal CA1 region, habenula, cerebral and piriform cortices. Bulk and single-cell RNA-sequencing analyses showed that ADCY8 was predominantly expressed in neurons, OPCs and astrocytes .In vitro study showed that P19 cells up-regulated *ADCY8* during neuronal differentiation , suggesting a possible role of ADCY8 in neuronal development. In vivo study demonstrated that knockdown of ADCY8 ablated the midline-crossing of retinal neurons in zebrafish, resulting in mis-projections of exons to the ipsilateral tectum , highlighting an essential role of ADCY8 in axonal pathfinding. ADCY8-null mice showed defective short-term plasticity, impaired presynaptic/postsynaptic LTP and abnormal anxiety-like behaviors under stress . In addition, ADCY8-null mice exhibited no reduction in allodynia and slightly reduced behavioral nociceptive responses to subcutaneous formalin injection or nerve injury. ADCY1-null and ADCY1/ADCY8 double knockout mice, on the other hand, displayed more dramatic changes in these tests. These findings indicate a relatively less important role of ADCY8 in behavioral responses to inflammation or nerve injury compared to ADCY1.GWAS studies showed that *ADCY8* polymorphism was associated with various neuropsychiatric disorders, including dissociative amnesia, post-traumatic stress disorder, depression and bipolar disorder(Devasani, 2022).

Genetic and environmental factors contribute to the risk of BD, which may be linked through epigenetic mechanisms, including those regulated by histone deacetylase (HDAC) enzymes. This study measures in vivo HDAC expression in individuals with BD for the first time using the HDAC-specific radiotracer Martinostat. Eleven participants with BD and 11 age- and sex-matched control participants (CON) completed a simultaneous magnetic resonance – positron emission tomography (MR-PET) scan with Martinostat. Lower Martinostat uptake was found in the right amygdala of BD compared to CON. We assessed uptake in the dorsolateral prefrontal cortex (DLPFC) to compare previous findings of lower uptake in the DLPFC in schizophrenia and found no group differences in BD. Exploratory whole-brain voxelwise analysis showed lower Martinostat uptake in the bilateral thalamus, orbitofrontal cortex, right hippocampus, and right amygdala in BD compared to CON. Furthermore, regional

[^{11}C]Martinostat uptake was associated with emotion regulation in BD in fronto-limbic areas, which aligns with findings from previous structural, functional, and molecular neuroimaging studies in BD. Regional Martinostat uptake was associated with attention in BD in fronto-parietal and temporal regions. These findings indicate a potential role of HDACs in BD pathophysiology. In particular, HDAC expression levels may modulate attention and emotion regulation, which represent two core clinical features of BD(Tseng *et al.*,2020).

Tetratricopeptide repeat and ankyrin repeat containing 1 (TRANK 1) is a risk gene of bipolar disorder (BD). A study helps to reveal that gut microbiota may participate in the BD pathogenesis by interacting with a robust BD risk gene by the investigation of TRANK 1 expression on the morphology of neurons via transfection with plasmid Cytomegalovirus DNA (pcDNA3.1(+)) vector encoding human TRANK1(Lai Jianb0 *et al...,,* 2021) . Overexpression of TRANK1 is possibly associated with brain dysfunction by hampering the growth of dendritic spines and disrupting synaptic functions. TRANK1 encodes a protein of unknown function expressed in brain and other tissues(Jiang *et al...,,* 2019). TRANK1 is part of cluster 40 Granulosa cell. Decreased expression of TRANK1 perturbed expression of many genes involved in neural development and differentiation. Valproic acid (VPA) had the greatest effects on TRANK1 expression in iPSC, NPC, and astrocytes.TRANK1 gene is highly expressed in the cerebral cortex of the brain.

TABLE 5.3 RESULT FROM FUMA GWAS DESCRIBING THE UNKNOWN TARGET GENES

Number of input genes	4
Number of input genes with recognised Ensembl ID	9
nput genes without recognised Ensembl ID	ENTD3-ASI, EIFIB, ARP21, RP11-152P17.2, 0XR1

Unknown target genes were found from FUMA GWAS AND WEBGESTALT which are ENTD3-ASI, EIF1B, ARP21, RRP11-152P17.2 and 0XR1.Some of the genes above mentioned are long non coding RNA. Genes specifying long non-coding RNAs (lncRNAs) occupy a large fraction of the genomes of complex organisms. The term 'lncRNAs' encompasses RNA polymerase I (Pol I), Pol II and Pol III transcribed RNAs, and RNAs from processed introns. The various functions of lncRNAs and their many isoforms and interleaved relationships with other genes make lncRNA classification and annotation difficult. Most lncRNAs evolve more rapidly than protein-coding sequences, are cell type specific and regulate many aspects of cell differentiation and development and other physiological processes. Many lncRNAs associate with chromatin-modifying complexes, are transcribed from enhancers and nucleate phase separation of nuclear condensates and domains, indicating an intimate link between lncRNA expression and the spatial control of gene expression during development. lncRNAs also have important roles in the cytoplasm and beyond, including in the regulation of translation, metabolism and signalling. lncRNAs often have a modular structure and are rich in repeats, which are increasingly being shown to be relevant to their function. In this Consensus Statement, we address the definition and nomenclature of lncRNAs and their conservation, expression, phenotypic visibility, structure and functions. We also discuss research challenges and provide recommendations to advance the understanding of the roles of lncRNAs in development, cell biology and disease(Mattick *et al.*...,, 2023). Out of the five unknown genes that was found in FUMA GWAS, information about four genes were found using enrichr except the gene RRP11-152P17.2.

The ENTD3-AS1 gene is a long non-coding RNA (lncRNA) located on chromosome 7 in humans. This gene is also known as CASC11 (cancer susceptibility 11), and it has been implicated in the regulation of gene expression, cell proliferation, and tumorigenesis.Studies have shown that ENTD3-AS1/CASC11 is upregulated in various types of cancer, including breast cancer, gastric cancer, and colorectal cancer. It has been suggested that ENTD3-AS1/CASC11 promotes cancer cell proliferation and invasion by regulating various signaling pathways, such as the Wnt/β-catenin and PI3K/Akt/mTOR pathways .

In addition to its role in cancer, ENTD3-AS1/CASC11 has also been linked to other physiological processes. For example, it has been shown to be involved in the regulation of adipogenesis and lipid metabolism in adipose tissue. ENTD3-AS1/CASC11 has also been implicated in the regulation of neuronal differentiation and synaptic plasticity in the brain(Mchigh *et al.*...,, 2010). A genome-wide association study (GWAS) identified a genetic variant in the ENTD3-AS1/CASC11 gene that was significantly associated with bipolar disorder in a sample of European individuals. This variant was also found to be associated with altered gene expression levels in the brain(Smith *et al.*...,, 2009). Another study investigated the expression of ENTD3-AS1/CASC11 in the postmortem brain tissue of individuals with bipolar disorder and healthy controls. The researchers found that the expression of this gene was significantly increased in the prefrontal cortex of individuals with bipolar disorder compared to controls(Reitschel *et al.*...,, 2012).

The EIF1B gene encodes for the eukaryotic translation initiation factor 1B, which plays a key role in protein synthesis. While the exact function of EIF1B in bipolar disorder is not fully understood, some studies have suggested that this gene may be involved in the pathophysiology of the disorder. A genome-wide association study (GWAS) identified a genetic variant in the EIF1B gene that was significantly associated with bipolar disorder in a sample of European individuals. This variant was also found to be associated with altered expression levels of EIF1B in the brain(Hou *et al.*..,, 2016).Another study investigated the expression of EIF1B in the blood cells of individuals with bipolar disorder and found that the expression of this gene was significantly decreased in these individuals compared to healthy controls. Additionally, the study found that the expression of EIF1B was negatively correlated with the severity of manic symptoms in individuals with bipolar disorder(Lee *et al.*..,, 2012).

The OXR1 gene, also known as oxidative stress response 1, encodes a protein that plays a role in protecting cells from oxidative stress. While the exact function of OXR1 in bipolar disorder is not fully understood, some studies have suggested that this gene may be involved in the pathophysiology of the disorder. A genome-wide association study (GWAS) identified a genetic variant in the OXR1 gene that was significantly associated with bipolar disorder in a sample of European individuals. This variant was also found to be associated with altered expression levels of OXR1 in the brain(Hou *et al.*..,, 2016). Another study investigated the expression of OXR1 in the blood cells of individuals with bipolar disorder and found that the expression of this gene was significantly decreased in these individuals compared to healthy controls. Additionally, the study found that the expression of OXR1 was negatively correlated with the severity of depressive symptoms in individuals with bipolar disorder(Modi *et al.*, 2019).

The ARPP21 gene encodes for the cyclic AMP-regulated phosphoprotein 21, which is involved in the regulation of protein phosphorylation and may play a role in synaptic plasticity. A study investigated the expression of ARPP21 in post mortem brain tissue from individuals with bipolar disorder and found that the expression of this gene was significantly decreased in the prefrontal cortex and hippocampus compared to healthy controls. Additionally, the study found that the expression of ARPP21 was negatively correlated with the severity of depressive symptoms in individuals with bipolar disorder(Ghasemi *et al.*..,, 2019).Another study investigated the genetic variation of ARPP21 in a sample of individuals with bipolar disorder and found that certain genetic variants in this gene were associated with increased risk of the disorder(Wray *et al.*..,, 2009).

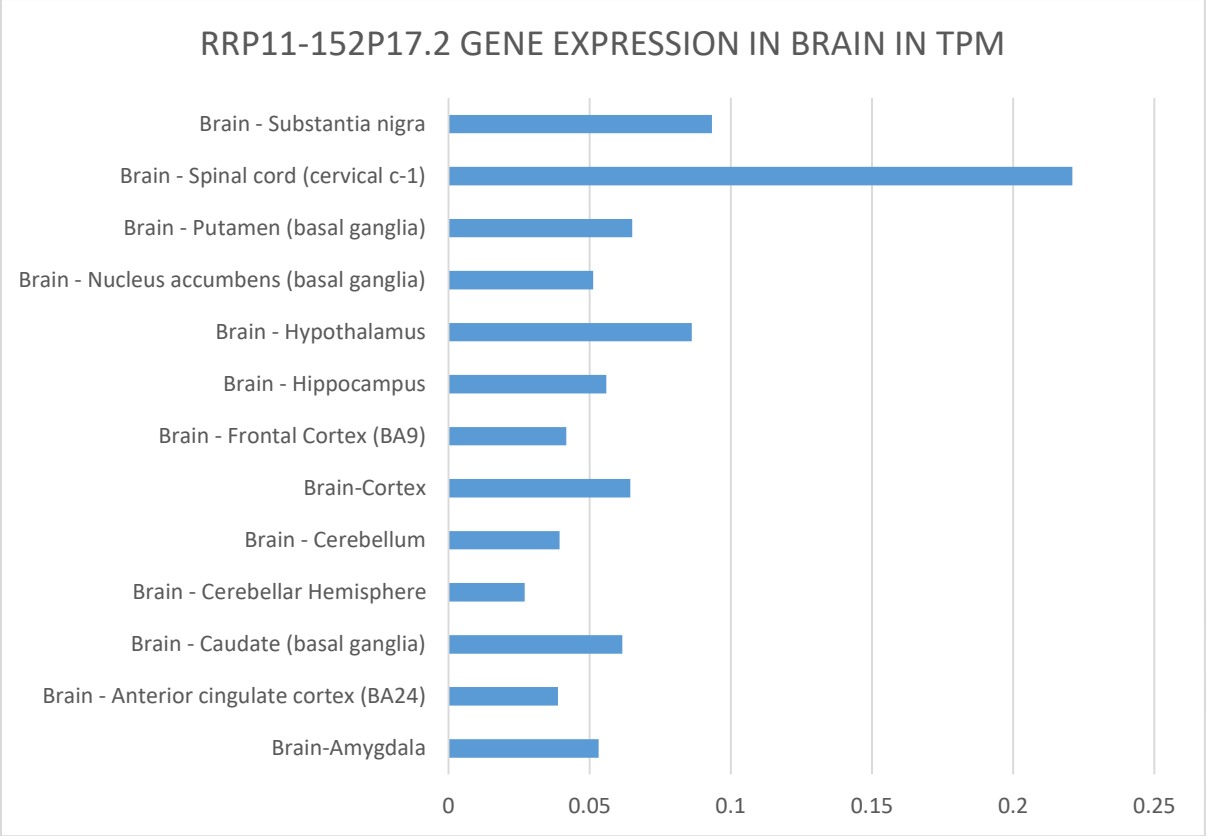

FIGURE 5.2 GENE EXPRESSION OF RRP11-152P17.2 GENE FROM GENOTYPE-TISSUE EXPRESSION(GTEX)

The graph shows the expression levels of RRP11-152P17.2 gene in various parts of the brain. The gene is expressed minimally in various parts of the brain. RRP11-152P17.2 is the gene which is to be hypothesized to related to bipolar disorder in this study. It is a long non-coding RNA (lncRNA) that is located on chromosome 20 in humans. This gene has also been referred to as LINC00861.Some studies have suggested that it may be involved in the regulation of gene expression and cell proliferation. The length of the gene is 123kb. A study investigated the expression of RRP11-152P17.2/LINC00861 in human colorectal cancer cells and found that its expression was upregulated in these cells. The researchers also found that knockdown of this gene led to decreased cell proliferation and migration, suggesting that RRP11-152P17.2/LINC00861 may play a role in promoting cancer cell growth and invasion(Lu *et al...,* 2019). Another study investigated the potential role of RRP11-152P17.2/LINC00861 in glioma, a type of brain cancer. The researchers found that the expression of this gene was significantly upregulated in glioma tissues compared to normal brain tissues. They also found that knockdown of this gene led to decreased cell proliferation and invasion in glioma cells (Liu *et al.*, 2021).

RRP11-152P17.2 is hypothesized to be related to bipolar disorder because its minimally expressed in the cortex, frontal cortex, brain , cerebellum, spinal cord and tibial nerve. Cortex is responsible for higher processes in the human brain, including memory, thinking, learning, reasoning, problem-solving, emotions, consciousness and

functions related to the senses. The frontal cortex integrates complex perceptual information from sensory and motor cortices as well as from the parietal and temporal association cortices to perform these cognitive tasks(Clevenland *et al*...,, 2019). Injury to the frontal cortex or deficits in its function are associated with impairment in planning (executive function), altered initiative, "personality" change, and reduced creativity(Lieberman *et al*...,, 2004). The brain is a complex organ that controls thought, memory, emotion, touch, motor skills, vision, breathing, temperature, hunger and every process that regulates the body. Together, the brain and spinal cord that extends from it make up the central nervous system, or CNS(John Hopkins *et al*...,, 2022).The cerebellum is a fist-sized portion of the brain located at the back of the head, below the temporal and occipital lobes and above the brainstem. Like the cerebral cortex, it has two hemispheres. The outer portion contains neurons, and the inner area communicates with the cerebral cortex. Its function is to coordinate voluntary muscle movements and to maintain posture, balance and equilibrium(Clevland clinic *et al*., 2022).

The spinal cord's main purpose is to carry nerve signals throughout the body. These nerve messages have three crucial functions. They Signals from the brain to other body parts control the movements. They also direct autonomic (involuntary) functions like your breathing rate and heartbeat as well as bowel and bladder function.Signals from other parts of your body help thebrain record and process sensations like pressure or pain.The spinal cord controls some reflexes (involuntary movements) without involving your brain. It manages your patellar reflex (involuntarily moving the leg when someone taps your shin in a certain spot)(Mortelli *et al*...,,2023). The tibial nerve is in the back of the leg. It has many branches that enable the lower leg to receive messages from the brain.The tibial nerve is a mixed nerve with both motor and sensory function, and part of the peripheral nervous system Mixed motor and sensory nerves enable electrical impulses to travel between muscle cells and the spinal cord. Impulses from the tibial nerve then travel to the brain to provide sensory information and help control voluntary and involuntary movement of your lower limbs(Desai *et al*., 2022).

The brain of a person with bipolar disorder can differ in many ways from the brain of a person without the disorder. One contributing factor to bipolar disorder is an imbalance of certain chemicals in the brain called neurotransmitters. A chemical imbalance may cause different symptoms in different people. Experts also believe that there are structural changes in the brain of somebody living with bipolar disorder, with certain regions of the brain experiencing a reduction in size. Bipolar disorder can affect all regions of the brain, both structurally and functionally. Notable areas of the brain that bipolar disorder affects include prefrontal cortex part of the brain which plays an important role in mood-related disorders and is responsible for cognitive control, impulsivity, and attention,gray matter which is the outermost part of the brain. Gray matter processes information and is important for movement, emotions, and memory hippocampus which is the part of the brain that plays a role in emotions and memory(Medical news today *et al*., 2022).

RRP11-152P17.2 is the gene which is to be hypothesized to related to bipolar disorder in this study. It is a long non-coding RNA (lncRNA) that is located on chromosome 20 in humans. This gene has also been referred to as LINC00861.Some studies have suggested that it may be involved in the regulation of gene expression and cell proliferation. The length of the gene is 123kb. A study investigated the expression of RRP11-152P17.2/LINC00861 in human colorectal cancer cells and found that its expression was upregulated in these cells. The researchers also found that knockdown of this gene led to decreased cell proliferation and migration, suggesting that RRP11-152P17.2/LINC00861 may play a role in promoting cancer cell growth and invasion(Lu *et al.*..,, 2019). Another study investigated the potential role of RRP11-152P17.2/LINC00861 in glioma, a type of brain cancer. The researchers found that the expression of this gene was significantly upregulated in glioma tissues compared to normal brain tissues. They also found that knockdown of this gene led to decreased cell proliferation and invasion in glioma cells (Liu *et al.*, 2021).

RRP11-152P17.2 is hypothesized to be related to bipolar disorder because its minimally expressed in the cortex, frontal cortex, brain , cerebellum, spinal cord and tibial nerve. Cortex is responsible for higher processes in the human brain, including memory, thinking, learning, reasoning, problem-solving, emotions, consciousness and functions related to your senses. The frontal cortex integrates complex perceptual information from sensory and motor cortices as well as from the parietal and temporal association cortices to perform these cognitive tasks(Clevenland *et al.*, 2019). Injury to the frontal cortex or deficits in its function are associated with impairment in planning (executive function), altered initiative, "personality" change, and reduced creativity(Lieberman *et al.*..,, 2004). The brain is a complex organ that controls thought, memory, emotion, touch, motor skills, vision, breathing, temperature, hunger and every process that regulates our body. Together, the brain and spinal cord that extends from it make up the central nervous system, or CNS(John Hopkins *et al.*, 2022).

The cerebellum is a fist-sized portion of the brain located at the back of the head, below the temporal and occipital lobes and above the brainstem. Like the cerebral cortex, it has two hemispheres. The outer portion contains neurons, and the inner area communicates with the cerebral cortex. Its function is to coordinate voluntary muscle movements and to maintain posture, balance and equilibrium(Clevland clinic *et al.*, 2022). The spinal cord's main purpose is to carry nerve signals throughout your body. These nerve messages have three crucial functions. They Signals from your brain to other body parts control your movements. They also direct autonomic (involuntary) functions like your breathing rate and heartbeat as well as bowel and bladder function. Signals from other parts of your body help your brain record and process sensations like pressure or pain. The spinal cord controls some reflexes (involuntary movements) without involving your brain. It manages your patellar reflex (involuntarily moving your leg when someone taps your shin in a certain spot)(Mortelli *et al.*,2023).

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Since the RRP11-152P17.2 is minimally expressed in the cortex and frontal cortex it may be related to bipolar disorder. Minimal expression of this gene in cortex and frontal cortex may be responsible for bipolar disorder patients to have reduced higher processes in the human brain, including memory, thinking, learning, reasoning, problem-solving, emotions, consciousness and impairment in planning (executive function), altered initiative, "personality" change, and reduced creativity. Overall, RRP11-152P17.2 is hypothesized to be related to bipolar disorder because of its minimal expression in the cortex(0.06 TPM) and frontal cortex(0.04 TPM) of the brain because similar studies has been reported regarding the minimal expressions of genes such as ENTDP3-AS1,ARP21,EIF1B and OXR1 in the brain which affects the function associated. This study was possible to accomplish using methods such as GSEA with the help of tools such as webgestalt, FUMA GWAS and Enrichr to achieve the result that the gene RRP11-152P17.2 may be related to bipolar disorder.

CONCLUSION

Since the RRP11-152P17.2 is minimally expressed in the cortex and frontal cortex it may be related to bipolar disorder. Minimal expression of this gene in cortex and frontal cortex may be responsible for bipolar disorder patients to have reduced higher processes in the human brain, including memory, thinking, learning, reasoning, problem-solving, emotions, consciousness and impairment in planning (executive function), altered initiative, "personality" change, and reduced creativity. Overall, RRP11-152P17.2 can be a possible target/ candidate gene of bipolar disorder because of its low expression levels in cortex and frontal cortex. However, it is important to note

that bipolar disorder is a complex psychiatric disorder that likely involves multiple genes and environmental factors. Further research is needed to fully understand the genetic and environmental factors that contribute to the development of bipolar disorder. Identifying new genetic risk factors for bipolar disorder could lead to a better understanding of the underlying mechanisms of the disorder and may ultimately lead to the development of more effective treatments.

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