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# Analysis of the Genome Sequence for the Purpose of Understanding the Causes and Treatments of Bipolar Disorder and Related Disorders

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**KEYWORDS**
BP; DSM; WHO; CNV; ANOVA.

**Abstract:** In cases of bipolar disorder (BD), genetic factors have only been shown to contribute to a minor portion of the condition's heritability. Because of this, people have been looking into sub-phenotypes of BD, such as treatment response, in an effort to narrow the heterogeneity of BD. Using a variety of methods, the researchers in this study conducted a series of experiments with the goal of identifying molecular signatures that are related to bipolar disorder (BD), the response to lithium medication, and other subclinical traits in a cohort of individuals who have BD. First, we investigated the connection between lithium response and essential candidate genes already known to influence lithium response in other populations. In the second step of the process, a global and CpG island DNA methylation profiling was carried out with the goal of locating genomic loci that had distinct methylation patterns in lithium responders as opposed to non-responders. Third, a genome-wide copy number variation (CNV) study was done to find and describe CNVs that are linked to BD symptoms and how well lithium works. Finally, the results of an untargeted plasma metabolomic profiling showed that patients with bipolar disorder, both those who responded to lithium treatment and those who did not, had different amounts of a number of different metabolite species. The study's results support the idea that BD is most likely caused by the dynamic dysregulation of a large number of gene regulatory networks, proteins, and metabolic pathways, which is a sign of complex problems in the system.

## 1. Introduction:

Formerly called manic-depressive disease, bipolar affective disorder is a neuropsychiatric condition currently categorized as a mood disorder. Patterns of aberrant and extreme mood swings, from periods of depression to heightened mood, which is called mania, characterize this medical disease for which this diagnostic word is used. Thereis a wide variety of manifestations of bipolar disorder (BD) [1]. Treatment success is condition-specific and can occur in tandem with other psychiatric disorders. Without treatment, BD can have devastating effects on not just the quality of life of those who suffer from it but also their loved ones and the larger community [2]. Psychosis, both manic and depressive, can occur in the most severe forms of BD, which is coupled with cognitive and behavioral issues. The disease is extremely problematic from a public health perspective because it leads to increased suicide mortality and requires recurrent hospitalization [3].

### 1.1. Nosology, Symptoms, and Clinical Course of BD:

Psychiatric conditions that fall under the umbrella of "bipolar disorder" are quite varied. Clinical studies that tracked patients over time in the late 20th century revealed a striking diversity of phenotypes among the affected group. Mania without depression, dysthymia, cyclothymia, and mania type I and II are all part of the bipolar spectrum. Figure 1 depicts some of the most

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common types of mood disorders and the symptoms associated with them [4]. BD is a chronic condition that can progress in a variety of ways throughout the course of a person's lifetime. The initial manifestation of BD might manifest as either manic or hypomanic symptoms, depressive ones, or a combination of the three. The initial episode of bipolar disorder is typically manic for men, while the first episode of depression is equally common in both sexes [5]. More than ten affective episodes may occur in a patient's lifetime if BD is left untreated. Most people with bipolar disorder have typical 'euthymic' intervals between manic and depressive episodes, during which they revert to a more or less normal mental state. In people with bipolar disorder, the duration of both manic and depressive episodes, as well as periods of remission (euthymia), varies widely. Manic episodes tend to be shorter in duration than those of depression or mania [6].

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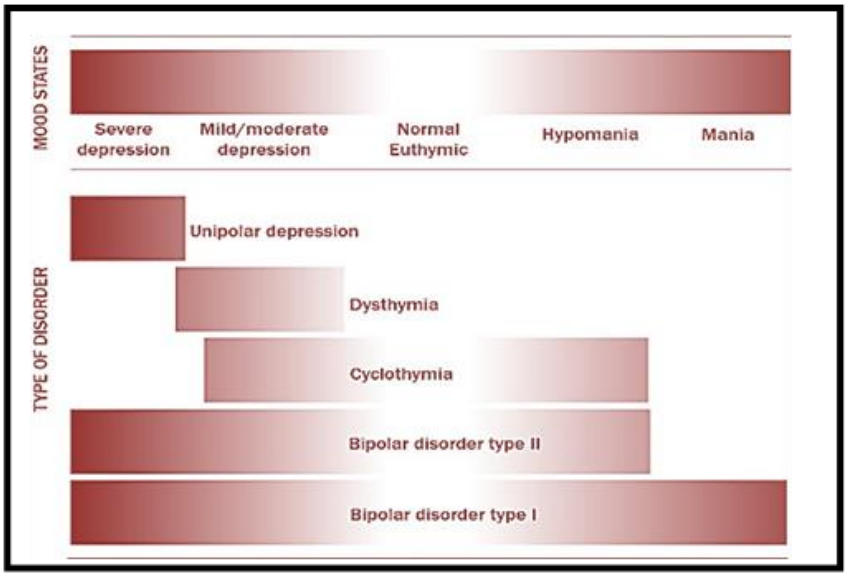


**Figure 1: Characteristics of mood states in different types of mood disorders.**

It has been estimated that the average duration of a manic episode is six weeks. It takes eleven weeks on average for a major depressive episode and seventeen weeks for a mixed episode. The time between episodes is shorter in later seasons compared to the first three. After the first three episodes, both the number of episodes and their length begin to reduce and increase gradually [7]. Rapid cycling describes patients who have more than four episodes of disturbed mood per year. Females with BD are more likely to experience rapid cycling, which affects 5-20% of adult females. Some people with BD have a regular, predictable pattern of episode recurrence, with shifts from depression to mania or mania to depression, whereas others have no such pattern [8]. The long-term effects of BD-I in a South Indian community were assessed in a recent survey. Mania is the most common and often the initial manifestation of bipolar disorder (85% of cases) [9].

Affective episodes, most frequently mania, accounted for 11% of patient lifetimes on average. The average length of a depressive or manic episode was two months, and the average length of time until a relapse was 21 months [10-11]. Similarly, a cohort from north-east India and research conducted in a rural Indian community both found that BD often manifested itself through episodes of recurring mania.

### 1.2 The Diagnostic Procedure

The diagnosis of BD, like that of other psychiatric diseases, is based on a thorough evaluation of the patient's psychological and behavioural health due to the lack of appropriate clinical laboratory tests. When diagnosing a patient with a mental disorder, psychiatrists follow established diagnostic interview methods that take into account the patient's history, personality, social and functional limitations, and other factors [12]. To rule out physical causes of psychiatric symptoms, it is occasionally necessary to perform a physical examination and review the patient's medical history. All diagnosable mental and behavioural illnesses are listed in chapter V of the tenth revision of the ICD (International Classification of Diseases). Diagnostic criteria for BD and related symptoms are detailed in the International Classification of Diseases, 10th Revision (ICD-10) (http://apps.who.int/classifications/icd/). The American Psychiatric Association's (APA) Diagnostic and Statistical Manual of Mental Disorders (DSM) is the gold standard for psychiatric diagnosis. Every few years, the International Classification of Diseases (ICD) and the Diagnostic and Statistical Manual of Mental Disorders (DSM) are updated or altered to reflect

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the latest research findings in the field of psychiatry. The following sections outline the key criteria used in the DSM-IV-TR to diagnose BD in clinical practice [12]

**1.3. Genetics:**

Many different clinical observations have been made in BD, but the most consistent one is that BD is passed down via families. This is why a large portion of current studies aim to identify risk factors for the condition, such as specific genes or chromosomal locations [13]. Genetic research in the 21st century has made great progress in understanding the biology of BD, even though the specific genes or chromosomal locations causing this condition are still obscure. Multiple physiological mechanisms and alterations in CNS structure and function have been related to genetic predispositions to BD. Additional features of BD that are compatible with epigenetic dysregulation include discordance in monozygotic twins, parent-of-origin effects (POEs), late age of onset, and a varied illness course [14]. Epigenetic processes have been the focus of recent research into BD. The literature review in this thesis provides additional information on the genetics and epigenetics of BD [15].

**2. Related work:**

Research conducted on families, twins, and adoptees over the past several decades has been crucial in elucidating the heritable nature of BD. It has been calculated via studies of families that the relative risk of BD among first-degree relatives is approximately 10%, or ten times that of the general population [16]. There is a 2-fold increase in the incidence of unipolar depression in first-degree relatives. In families with a history of bipolar disorder, unipolar depression is more prevalent due to its high population incidence. Moreover, schizoaffective disorder and schizophrenia were also found to occur at elevated rates in bipolar families. Diverse families have different histories, making it difficult to generalize about the transmission of BD. The true nature of heredity is further muddled by the existence of other psychiatric diseases in families where BD is present [17].

If one twin has BD, there is a 70-85% chance that the other does as well; for dizygotic twins, the risk is 15-25%. Takinginto account these variations, BD has one of the highest estimated heritabilities among all psychiatric diseases, at 70–85 percent. As a result, environmental impacts, such as psychological factors, account for some of the risk (15-20%), meaning that it is not entirely genetic [18]. Research on adopted people with BD has found that their adoptive families tend to have fewer members with the disorder than their biological families. These findings provide more proof that BD is a genetically predisposed psychiatric disorder.

Candidate genes for neuropsychiatric diseases are those that code for critical enzymes in neurotransmitter metabolism [19]. The enzyme catechol-O-methyltransferase (COMT) is involved in the breakdown of the neurotransmitter catecholamines. The amino acid valine 158 is replaced by methionine as a result of the single-nucleotide polymorphism (SNP) rs4680 (A/G), also called Val158Met, in the COMT gene [20-22]. It has been found that the Val allele variant catabolizes dopamine at a rate roughly four times faster than its Met equivalent. Dopaminergic neuronal activity and synaptic dopamine levels are both raised when the Met allele slows down the pace at which dopamine is broken down. Both schizophrenia and BD, in which there is an excess of dopamine in the brain, have been linked to the Met allele. Several other COMT polymorphisms, including Val158Met, have been associated with pharmacological response, especially to second-generation antipsychotics that target the dopaminergic system [23]. Serotonin and other serotonin- related neurotransmitters are synthesized in part by the enzymes tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH1 and TPH2). Multiple studies have linked genetic variations in these genes to BD and schizophrenia. Evidencefor BD has also been found in the genes D-amino acid oxidase (DAO), D-amino acid oxidase activator (DAOA), and monoamine oxidase A (MAOA). The vast majority of antipsychotic medicines work

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by blocking the effects of certain neurotransmitters at their receptors. Multiple studies have looked into the possible significance of neurotransmitter receptor genes in BD's genesis and therapeutic response. Multiple studies corroborated the findings in favor of the dopamine DRD3 and DRD4 receptors and the serotonin HTR2A and HTR2C receptors [24-27]. Furthermore, recent research has indicated a substantial relationship between BD and genetic variations in NMDA glutamate receptors and numerous types of GABA receptors. One of lithium's best-studied targets is glycogen synthase kinase 3 (GSK3). Although early reports suggested a positive relationship between SNPs in this gene and BD, several contradictory studies have since been published [28].

### 2.1. Psychotic Symptoms:

Some people with BD also have psychotic symptoms of schizophrenia, such as delusions and hallucinations. The psychotic episode may accompany mania or depression. Throughout the course of their illness, more than half of BD patients will have a psychotic episode at some point. The most typical sign of psychosis is grandiose delusions, but others include hallucinations, inconsistent moods, and cognitive instability [29]. Patients with BD who also exhibit psychotic symptoms may constitute a distinct subtype of the condition, one that shares common etiological roots and genetic vulnerability with schizophrenia. The genetic analysis of psychotic BD is gaining attention as multiple studies demonstrate that psychotic characteristics tend to cluster in BD families [30]. Several chromosomal areas have been linked to potential psychotic BD susceptibility genes by linkage studies. COMT has been linked to this condition in candidate gene association studies.

### 2.2. Family History:

One of the most reliable predictors of increased risk for BD is a family history of mood disorders. Family studies of BD have shown that a child with even one parent who also suffers from a mood illness has a 10-25% chance of developing the same condition. It nearly doubles if both parents are affected. Children are more likely to contract an illness if they have a large number of affected relatives [31-33]. First-degree relatives, as opposed to more distant relatives, carry a higher risk. Patients with BD who have a high prevalence of psychiatric illness in their families likely have a heavier burden from one or more causative genetic variations. Patients with BD, whether they have a family history or not, are just at the start of a comprehensive genetic analysis.

### 2.3. Genomic Structural Variations:

Population and medical geneticists have focused extensively over the past few decades on identifying and mapping variants in the human genome. Short genetic variants and structural variants make up the bulk of human genomic variability [34]. Common polymorphisms and point mutations are examples of the short-range genetic variants that make up the vast majority of the human genome. This category also contains repeat polymorphisms with a length of less than one kilobase (STRs, short tandem repeats), as well as shorter insertions and deletions.

VNTRs (variable-length polymorphic tRNA-derived nucleotide repeats; di, tri, tetra, etc. Genomic variants that affect regions of DNA larger than one kilobase (kb) are classified as structural variations (SVs). SVs can range in size from very small to microscopic and encompass a wide variety of alterations, including copy [35]. Changes in copy number, including additions, deletions, switches, and transpositions.

Copy number variation refers to the quantitative difference between the copy number of DNA at a given genomic region of size greater than 1 kb and a reference genome of choice and can take the form of gain (insertion, duplication/amplification), loss (deletion), or null genotype (CNV). A common nuclear variation in copy number, polymorphisms are mutations that occur at a rate of more than 1% in any given population (CNP) [36]. Segmental duplications are regions of DNA larger than 1 kb that is repeated several times in the genome with higher than 90% sequence identity. Approximately, duplications the extent of segmental duplications

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varies from 1 to 14 percent across the 24 chromosomes, making up 4 percent of the human genome. CNVs can come in many forms, from simple duplications and deletions to more complicated inversions and translocations, depending on the method by which they were created [37]. Changes in copy number can influence gene expression in ways apart from gene dosage and long- term effects on global gene expression, including reshaping chromatin structure and affecting the function of gene regulatory and enhancer elements.

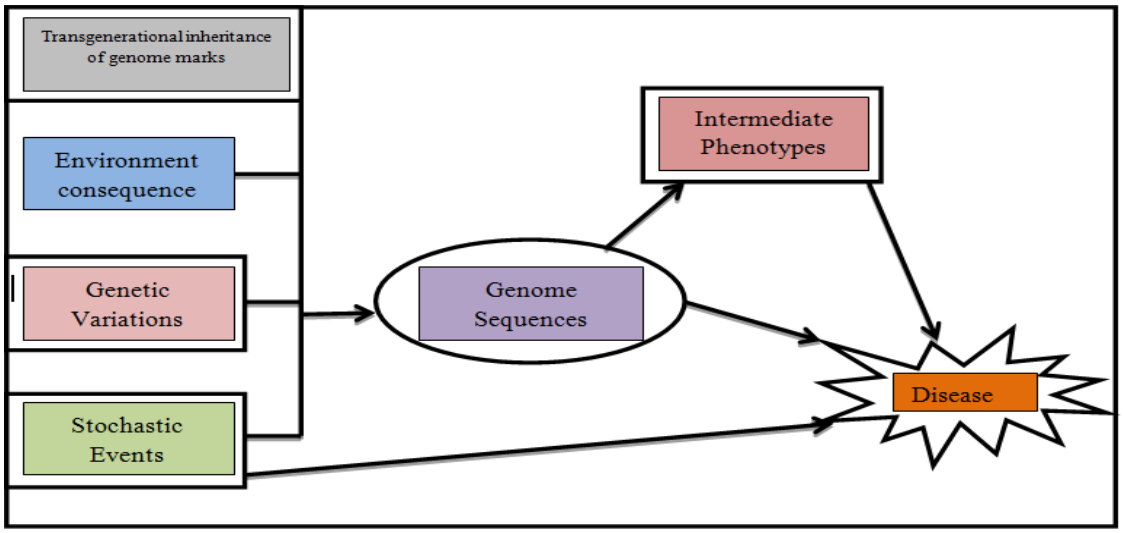


**Figure 2: Inter-relationships among the epigenetic modification and other causal factors in complex psychiatricdisorders.**

DNA obtained from peripheral leukocytes or post-mortem brain tissue was employed for the majority of the earliest epigenetic investigations in neuropsychiatric illnesses, which mostly focused on the examination of epigenetic marks in specific candidate genes. DNA methylation variations in catechol-O-methyltransferase (COMT) and reelin (RELN) have been linked to BD and schizophrenia in studies using methylation-specific polymerase chain reactions [38]. These results were not confirmed, however, by subsequent research utilizing more sensitive quantitative approaches such as bisulfite pyro-sequencing. An increased concentration of SAM and increased levels of DNMT1 gene expression were found in the brains of people with schizophrenia and BD. They also discovered that reelin and GAD67 gene mRNAs were down-regulated in cortical GABAergic neurons due to elevated promoter methylation. Down-regulation of DNMT1 and DNMT3A mRNA was later shown to be upregulated in peripheral blood cells [39-40]. No major alterations in global methylation were found in brain samples or peripheral leukocytes, despite the finding of DNMT up-regulation in psychosis.

## 3. The objective of this research:

This research was set up so that scientists could get a good grasp on how numerous genetic events contribute to the phenotype of lithium receptivity in people with BD. This was done on the premise that evidence for the additional study could be found in the integration of many genetic abnormalities, as is possible in complex illnesses like BD. Because of this, the following are our detailed goals. Genome-wide aCGH strategy to identify and validate genomic regions/genes with copy number variation (CNVs) in individuals with BD, and to correlate such CNVs with the lithium response phenotype and clinical subtypes of BD. Analysis of plasma

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metabolites in a group of bipolar disorder patients defined by their level of responsiveness to lithium.

## 4. Proposed Algorithm:

The proposed flow diagram is shown below in figure 3.

### 4.1. Bipolar Disorder cases:

After a thorough mental health evaluation, review of case records from hospital outpatient departments, and in-person interview, a diagnosis of BD was made using the DSM IV-TR criteria (boxes 1, 2, 3, and 4). The psychiatrists evaluated and diagnosed all of the patients. Specifically, a young psychiatrist first assessed the patients and meticulously recorded their mental and clinical histories. A prominent psychiatrist oversaw the final assessment and treatment plan. Individuals were selected on the basis that they had BD as their primary illness, the one that caused the most substantial discomfort or dysfunction, and so led them to seek therapy. The vast majority of BD patients (98.5%) were classified as having type I BD. Head trauma, major physical or neurological illness, substance use disorders, and mental retardation were also ruled out because of their correlation to neuropsychological impairment but were not included. We excluded patients who had been diagnosed with multiple Axis-I diseases.

### 4.2. Lithium Response Analysis:

Psychiatrists looked back at their patients' clinical symptoms while they were on long-term lithium medication to determine how well it was working. Throughout the acute stages of their illness, patients were seen once every week and once every three months during remission. Patients who experienced a recurrence of major depression or mania following a euthymic period of at least six months were diagnosed with a new episode and given additional care (hospitalization, medication change if necessary) and treatment based on the clinician's assessment of the situation. Minor depressive episodes and other subclinical presentations were not considered new recurrences. Our routine clinical treatment intervention and monitoring of the patient's illness progression was provided to all patients. There was no use of ECT or other psychiatric treatments. Following the best guess approach, we interviewed patients, family members, and previous health professionals and obtained records to learn as much as we could about their sickness before they contacted our center. For the therapeutic benefit of lithium medication to be evaluated, a minimum follow-up period of two years was recommended. Calculating plasma lithium levels in the clinical biochemistry lab was used to evaluate KH patients' adherence to lithium therapy. Levels of lithium in the plasma were monitored at least once every three months, and values between 0.6 and 1.2 mmol/L were regarded as having therapeutic value.

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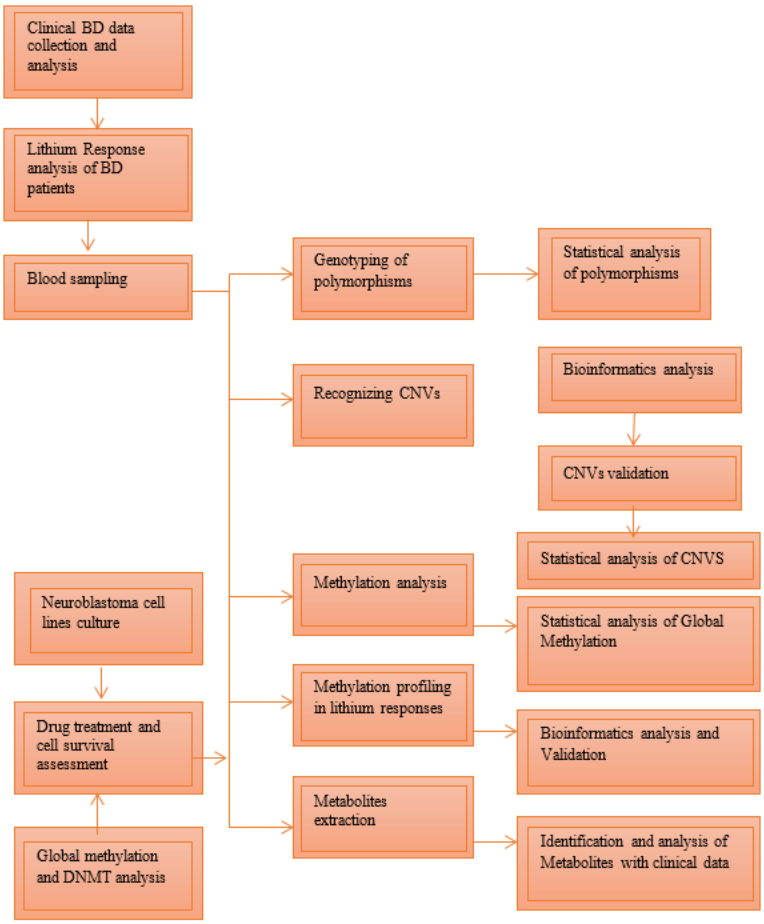


**Figure 3: The proposed flow- diagram.**

### 4.3. Genotyping of polymorphisms:

The samples were genotyped for the single-nucleotide polymorphism (SNP) rs25531 within the 5-HTTLPR, which is a 44-bp insertion-deletion polymorphism. We employed a PCR test to analyze rs25531 for both ins/del polymorphism and genotype. A pair of primers (forward: 5'-GCCAGCACCTAACCCCTAAT-3'; reverse: 5'-AGGGGATCCTGGGAGAG3') were chosen to amplify a 249 bp area surrounding the insertion, as was previously described. In a 25 ul reaction containing 1X PCR buffer, 100 ng of each primer, 1 unit of Taq DNA polymerase, and100 M of each dNTP, the target sequence was amplified from 100 ng of genomic DNA. Initial denaturation at 95 degrees Celsius for 5 minutes was followed by 35 cycles at 95 degrees Celsius for 30 seconds, 60 degrees Celsius for 45 seconds, and 72 degrees Celsius for 1 minute. Electrophoresis on a 1.5% agarose gel stained with ethidium bromide and having a DNA ladder of 100 bp allowed for the separation of the amplification products. Two types of alleles, S (206 base pairs) and L, were identified (249 bp). The MspI restriction endonuclease (CCGG) uses the SNP as part of a recognition site, cutting the site when the G nucleotide is present but leaving it uncut when the A nucleotide is present. Overnight MspI digestion of PCR results provides a 249 bp fragment (uncut LA allele), two pieces of 148 bp and 101

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bp (cut LG allele), or a 206 bp fragment (S allele). Protein fragments were isolated on ethidium bromide-stained 2% agarose gels. Sanger sequencing was used to confirm the rs25531 genotypes of a subset of samples.

**4.4. Identification of CNVs by array CGH analysis:**

Agilent feature extraction software was used to examine microarray pictures (protocol CGH-v4 91). Based on the array format, a microarray image-aligned grid template was chosen. The interactive adjust-corners algorithm uses an array of dark spots to optimize grid alignment. Cookie-cutter algorithm is used to detect spots and calculate local background intensities surrounding each spot for background removal. Outlier pixels were separated from designated pixel populations. Featured pixels were saturated. Each spot's pixel intensities' mean, median, and standard deviation were calculated. The error was calculated for each characteristic using a universal model. Multiplicative detrending removes non-systematic array intensity distribution. A dye normalization curve fit determined differences in red and green channel intensities induced by labelling and/or fluorescence emission. The log2 ratio of red and green intensities was then calculated. Detecting genomic copy number abnormalities requires measuring array log ratio noise. Calculating the robust standard deviation of log ratio differences between consecutive probes (dLRsd) along all chromosomes gives a reasonable assessment of noise. Signal intensities, background noise, and the signal-to-noise ratio were used to determine the best hybridization and washing settings.

The feature extraction data file was imported into Agilent's CGH module. CNV increases and losses were discovered using the conventional ADM-2 technique. This technique detects all anomalous intervals in a given sample with consistently high or low log ratios. Log2 ratios in at least two consecutive array probe signals were used to identify CNVs. Aberration zones are represented as a sample-colored bar graph. The ADM-2 algorithm with 6 thresholds discovered aberrant areas. Centralization method set at 6.0 and 10 bins. The minimum average log ratio for a region is 0.25, and the minimum number of probes in an aberrant interval is 3. CNV areas were visualized in genomic and chromosomal views along with log ratios.

**4.5. Bioinformatic Analysis:**

From the Genomic software workbench, we exported a list of CNV regions and associated metadata. Galaxy genome browser capabilities allowed for a combined study of CNV regions found in both responders and non-responders.

The CNV areas shared by responders and non-responders were divided into those that overlapped and those that did not, creating two distinct groups. Each group's shared and distinct areas were compared to the CNVs found in the general population and stored in the DGV database. The overlapped and separate CNV regions were shown in Venn diagrams. In addition, the coding genes (RefSeq), segmental duplications, repetitive elements, and CpG islands in the hg19 assembly were all mapped to CNVs. DAVID bioinformatics resources were used for the gene ontology analysis. Data from the Genetic Association Database (GAD), the CNVD database, Decipher, and BDgene were used to look for evidence of illness association in gene mapping inside the discovered CNV areas.

**4.6. CNVs validation and statistical analysis:**

The real-time polymerase chain reaction has surpassed other methods for detecting and quantifying DNA and RNA in recent years. One common technique for identifying CNVs is real-time quantitative polymerase chain reaction (RT-

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qPCR). At the end of each PCR cycle, the amount of DNA is quantified using fluorescent dyes that produce a rising fluorescent signal in direct proportion to the number of PCR amplicons created. While in the exponential phase, enough amplified DNA product builds up to provide a fluorescent signal. The CT represents the critical cycle in which this occurs. CT values are inversely proportional to the initial amount (in copies) of template present in a reaction because they are measured during the exponential phase when the reaction components are not constrained. By comparing the CT value of the unknown sample to that of a reference sample with a known copy number, it is possible to get an estimate of the copy number of the unknown sample.

### 4.7. Methylation statistical analysis:

Agilent's e-array database was queried for the probe sequence descriptions. The DMRs' whole sequences were mapped and annotated in the galactic genome browser. Promoters, CGIs, genes, coding and non-coding sections, distance from the transcription start site (TSS), untranslated regions (UTRs), and other genomic components were mapped to the DMRs. EpiExplorer was used to compare data from the ENCODE research on different human tissues and cell types with the DMRs.

The extent of methylation variation at individual gene promoters was determined by downloading promoter sequences from the DBTSS and mapping them to the DMRs. The methylation difference in a given genomic region was visualized using UCSC genome graphs. Genes serving as DMRs were subjected to functional analyses with KEGG and DAVID bioinformatics tools, including gene ontology, pathway enrichment, and disease connection. Tissue-specific gene expression data from the GEO library was then compared to the DMR-related genes. Based on their levels of expression in the body's blood tissue, hypermethylated and hypomethylated genes were separated.

## 5. Result and Discussion:

Based on the response criteria, 63 of the 203 BD patients enrolled in the current trial were designated, lithium responders. Clinical subjects were gathered from a single large mental healthcare center to ensure homogeneity in diagnostic procedures and lithium response evaluation. Thirty-one patients did not fulfill all inclusion criteria and, as a result, could not be assigned to a separate response group. Major factors contributing to excluding such patients for the assessment of lithium response included discontinuation of lithium before two years due to poor tolerance, side effects, intervening contraindications, poor therapeutic adherence to lithium, and inadequate information regarding the treatment outcome.

We compared demographic and clinical variables between the two groups to assess how they might have affected the subjects' lithium response outcomes in BD. Clinical and demographic data of bipolar disorder patients, lithium responders, non-responders, and controls were described using frequencies and percentages. In order to determine if there was a statistically significant relationship between the categorical variables, a Fisher's exact test was performed, whereas t rest was used for the continuous data. Patients had been suffering from the disease for a mean of 14 years and had suffered through a total of three abnormal mood episodes. Among these patients, manic episodes were the most common and contributed to the vast majority of hospital hospitalizations. Both the frequency of episodes and the prevalence of psychotic characteristics were greater in lithium non-responders than in responders (mean difference =

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0.12, 95% CI 0.09 to 0.33). No statistically significant variations in allele or genotype frequencies were found when comparing STin2 VNTR and 5-HTTLPR for all the clinical variables (table 1).

Where, $A_{i,k}$ is data and $A_k$ bar is centre value of data.

$$P - value = 10.log \frac{Max\ i}{\sqrt{MSE}}$$

The $X^2$ and P-values are calculated by following formula.

$$\frac{X^2}{m} = \frac{1}{} (A_{i,k} - A_k)^2$$

(2)

10

**Table 1: Comparison of STin2 VNTR and 5-HTTLPR for different clinical variables.**

| Clinical Covariate | Clusters | HTTLPR Alleles | | | VNTR alleles | $X^2$ Value | P-Value |
|---|---|---|---|---|---|---|---|
| | | S | $L_A$ | $L_G$ | | | |
| **Gender** | Males (65) | 0.63 | 0.25 | 0.10 | 0.71 | 0.98 | 0.91 |
| | Females (57) | 0.60 | 0.27 | 0.12 | 0.66 | | |
| **Age at onset ofBD** | Early (39) | 0.61 | 0.24 | 0.14 | 0.69 | 3.81 | 0.87 |
| | Intermediate (67) | 0.60 | 0.28 | 0.11 | 0.67 | | |
| | Late (16) | 0.71 | 0.21 | 0.06 | 0.78 | | |
| **Psychotic features** | Present (54) | 0.56 | 0.27 | 0.15 | 0.64 | 6.01 | 0.19 |
| | Absent (68) | 0.66 | 0.25 | 0.08 | 0.72 | | |
| **Thyroid abnormalities** | Present (23) | 0.54 | 0.28 | 0.17 | 0.67 | 2.48 | 0.64 |
| | Absent (99) | 0.64 | 0.25 | 0.10 | 0.69 | | |
| **Suicide attempts** | Attempted (16) | 0.71 | 0.15 | 0.12 | 0.71 | 2.27 | 0.68 |
| | Not attempted (106) | 0.60 | 0.27 | 0.11 | 0.68 | | |
| **Family history of psychiatric disorders** | Present (73) | 0.60 | 0.26 | 0.13 | 0.67 | 1.38 | 0.84 |
| | Absent (49) | 0.65 | 0.25 | 0.09 | 0.71 | | |
| **Psychosocial factors** | Present (26) | 0.67 | 0.26 | 0.05 | 0.73 | 2.60 | 0.62 |
| | Absent (96) | 0.60 | 0.26 | 0.13 | 0.68 | | |

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Genetic association studies on SLC6A4 polymorphisms and lithium responsiveness have been published, albeit with mixed results. These studies are predicated on the idea that the total activity and integrity of the serotonergic system are crucial for the action of lithium. S/S genotype has been linked to poor response to lithium in bipolar disorder and unipolar depression; however, the research is limited. Lithium prophylaxis has been demonstrated to be more effective in patients with the S/S genotype than in those with other genotypes, according to other research. An SNP (rs25531

A/G) within the 5-HTTLPR insertion has recently been found to be functionally triallelic, resulting in two variants of the L allele, LG and LA. Other results imply that rs25531 is located upstream of the 5-HTTLPR gene, but its precise mapping is still up for debate.

We have looked into the GADL1 SNPs rs17026688 and rs17026651 for any potential relationship with lithium responsiveness in BD. In our lab, a tetra-ARMS-based assay was used to identify the variant alleles. Allele- representative samples were Sanger sequence-verified, and those samples were used as positive controls in future PCR runs. Finally, the tetra-ARMS-determined genotypes were shown to be consistent with the genotypes of many randomly selected samples after validation through Sanger sequencing.

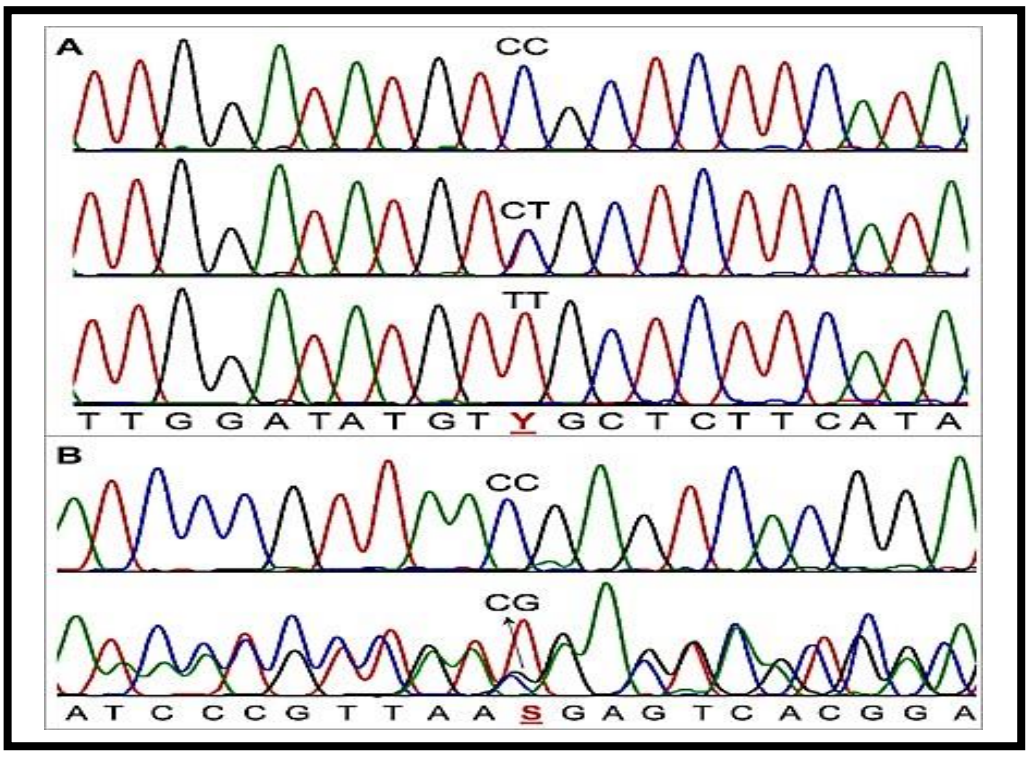


**Figure 4: Sanger sequencing electropherograms of samples representing different genotypes.**

CNV Identification: Agilent 244k (two-color) microarray aCGH analysis was used to determine genomic copy number gains and losses in BD lithium responders and non-responders. Two pools of samples, one from respondents and one from non-respondents, were hybridized into an array, and the third pool of DNA from a shared control group was used as a reference (R vs. C and NR vs. C). The Agilent 244k aCGH platform has 8.9 kb of total median probe spacing, 7.4 kb in coding areas, and 236,381 unique human genomic sequences annotated against NCBI Build 36 (UCSC hg18)

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**Table 2: CNVRs Count identified in the aCGH analysis**

| Group | CNV Types | Total number of CNV regions | Total size in Mbp |
|---|---|---|---|
| Non-responders | Gain | 165 | 67.43 |
| | Loss | 27 | 48.15 |
| Responders | Gain | 69 | 21.392 |
| | Loss | 90 | 10.12 |

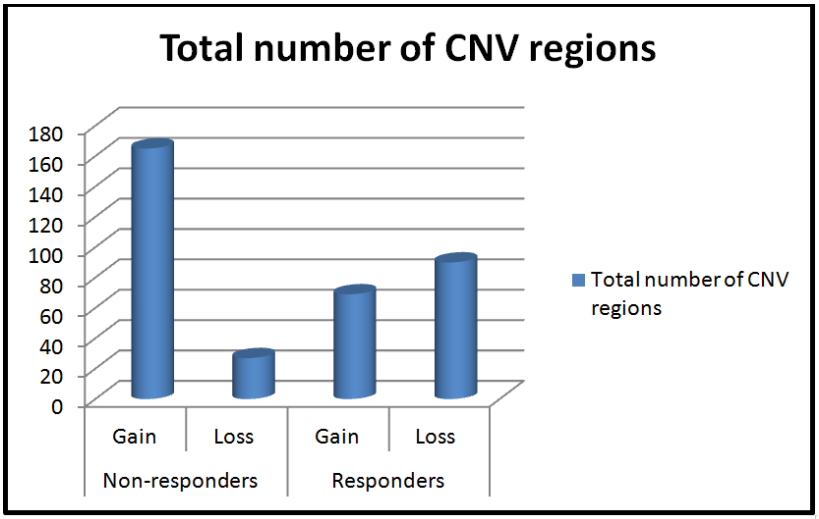


**Figure 5: Total number of CNV regions for Responder and Non-responder type CNVs.**

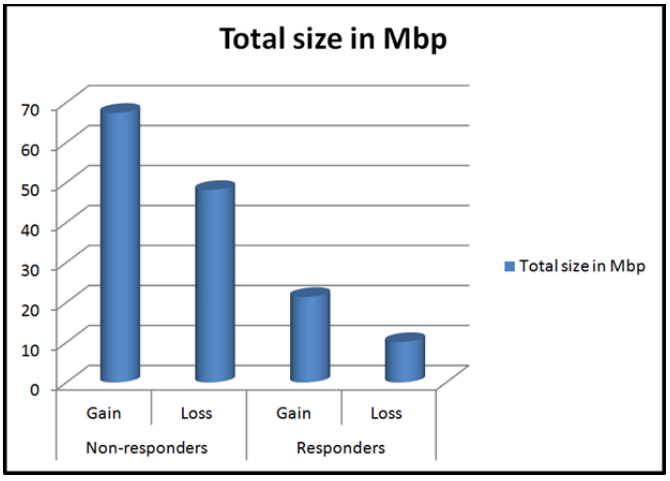


**Figure 6: Total size in Mbp for Responder and Non-responder type CNVs.**

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We analysed the genomic intervals of CNVs in responders and non-responders to identify the distinct regions affected in each group. Results showed that both responders and non-responders shared 34 CNV areas (31 gains and 3 losses). It's possible that the areas related to BD, rather than the lithium response, are represented by these frequent CNVs. We also tested the opposite, with CNV losses in non-responders vs growth in responders. We did not, however, uncover any CNVs of this common but opposite type.

## 6. Conclusion and Future Scope:

### 6.1. Conclusion:

Dynamic dysregulation of numerous gene regulatory pathways, proteins, and metabolic networks, reflecting complex perturbations of the system, is likely to result in neuropsychiatric illnesses like BD. Multiple methods were employed in this study to discover molecular markers linked with bipolar disorder, lithium responsiveness, and other subclinical traits in a group of BD patients from India. Patients diagnosed with primary BD had their responses to lithium medication retrospectively classified, yielding 103 non-responders and 62 responders.

Subjects with BD were classified according to a set of subclinical symptoms, including the presence of psychosis, a familial history of psychiatric disorders, suicidal behavior, etc. When comparing clinical features, the rate of suicidal behavior was much higher in lithium non-responders than in responders. Multiple studies have pointed to the widespread presence of CNVs in the human genome, raising the possibility that they play a significant role in neuropsychiatric illness susceptibility. Most genome-wide CNV investigations in BD have found no evidence of a greater CNV burden in BD patients than in healthy controls. Concurrent nucleotide variants (CNVs) may represent a unique genetic determinant for susceptibility to illnesses with a more prominent neurodevelopmental component than BD. It was found in our study that the frequency of CNVs larger than 1 Mb was substantially higher in non-responders than in responders. Our examination of CNV distribution and average size suggests that lithium non-responders have a higher CNV burden than responders. This study lends credence to the theory that individuals who do not respond to lithium have a more severe type of BD with deeper neurological and/or neurodevelopmental roots.

### 6.2. Future Scope:

The results provided in this paper represent one of the first attempts to throw light on the potential involvement of CNVs and epigenetic variables in BD, its subclinical manifestations, and the lithium response, although more research is needed to identify the actual function of the molecular markers revealed. Because of our limited knowledge of the inheritance process at work in complex illnesses and the nature of biological variation that might lead to disease, studying the aetiology of psychiatric disorders remains a difficult research subject. The ever-improving state of the art in high-throughput DNA sequencing technologies holds great hope for the discovery of new genetic variations linked to psychiatric disorders. The discovery of the genetic basis of psychiatric diseases is hoped to benefit greatly from large- scale whole-genome sequencing.

**References:**

1. Abdolmaleky HM, Yaqubi S, Papageorgis P, Lambert AW, Ozturk S, Sivaraman V *et al*. 2011. Epigenetic dysregulation of HTR2A in the brain of patients with schizophrenia and bipolar disorder. Schizophrenia research 129(2-3):183-190.
2. Adalsteinsson BT, Gudnason H, Aspelund T, Harris TB, Launer LJ, Eiriksdottir G *et al*. 2012. Heterogeneity in white blood cells has potential to confound DNA methylation measurements. PLoS One 7(10):e46705.
3. Adis medical writers. 2010. Lithium is still a first-line option in the treatment of patients with bipolar disorder. Drugs & therapy perspectives 26(3):9-14.
4. Aguilera O, Fernandez AF, Munoz A, Fraga MF. 2010. Epigenetics and

environment: a complex relationship.Journal of applied physiology 109(1):243-251.

5. Ahlawat S, Sharma R, Maitra A, Roy M, Tantia MS. 2014. Designing, optimization and validation of tetra-primer ARMS PCR protocol for genotyping mutations in caprine Fec genes. Meta Gene 2:439-449.
6. Alda M. 2015. Lithium in the treatment of bipolar disorder: pharmacology and pharmacogenetics. Molecular psychiatry 20(6):661-670.
7. Arts B, Simons CJ, Drukker M, van Os J. 2013. Antipsychotic medications and cognitive functioning in bipolar disorder: moderating effects of COMT Val108/158 Met genotype. BMC psychiatry 13:63.
8. Asai T, Bundo M, Sugawara H, Sunaga F, Ueda J, Tanaka G *et al*. 2013. Effect of mood stabilizers on DNA methylation in human neuroblastoma cells. International journal of neuropsychopharmacology 16(10):2285-2294.
9. Badner JA, Koller D, Foroud T, Edenberg H, Nurnberger JI, Jr., Zandi PP *et al*. 2012. Genome-wide linkage analysis of 972 bipolar pedigrees using single-nucleotide polymorphisms. Molecular psychiatry 17(8):818-826.
10. Baldessarini RJ, Faedda GL, Offidani E, Vazquez GH, Marangoni C, Serra G *et al*. 2013. Antidepressant- associated mood-switching and transition from unipolar major depression to bipolar disorder: a review. Journal of affective disorders 148(1):129-135.
11. Bannister AJ, Kouzarides T. 2011. Regulation of chromatin by histone modifications. Cell research 21(3):381- 395.
12. Beech RD, Leffert JJ, Lin A, Sylvia LG, Umlauf S, Mane S *et al*. 2014. Gene-expression differences in peripheral blood between lithium responders and non-responders in the Lithium Treatment-Moderate dose Use Study (LiTMUS). Pharmacogenomics journal 14(2):182-191.
13. Bergen SE, O'Dushlaine CT, Ripke S, Lee PH, Ruderfer DM, Akterin S *et al*. 2012. Genome-wide association study in a Swedish population yields support for greater CNV and MHC involvement in schizophrenia compared with bipolar disorder. Molecular psychiatry 17(9):880-886.
14. Beurel E, Grieco SF, Jope RS. 2015. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. Pharmacology and therapeutics 148:114-131.
15. Bigdeli TB, Ripke S, Bacanu SA, Lee SH, Wray NR, Gejman PV *et al*. 2015. Genome-wide association study reveals greater polygenic loading for schizophrenia in cases with a family history of illness. American journal of medical genetics part B neuropsychiatric genetics 171(2):276-89.