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Understanding The Causes And Treatments Of Bipolar Disorder And Related Disorders Through Genome Sequence Analysis

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Abstract:
It has only been demonstrated that a small percentage of the heritability of bipolar disorder (BD) is due to genetic factors. This has led to an attempt to reduce the heterogeneity of BD by focusing on sub-phenotypes of BD, such as treatment response. The objective of this study's investigations was to find molecular signatures associated with bipolar disorder (BD), the response to lithium treatment, and other subclinical characteristics in a cohort of BD patients using a range of techniques. Initially, we looked into the relationship between the reaction to lithium and crucial candidate genes that have previously been found to affect the response to lithium in different populations. To identify genomic regions that showed different methylation patterns in lithium responders compared to non-responders, a global and CpG island DNA methylation profiling was done in the second step of the approach. Third, to identify and characterize CNVs associated with both the effectiveness of lithium and the symptoms of BD, a genome-wide copy number variation (CNV) research was conducted. Ultimately, an untargeted plasma metabolomic profiling study revealed that patients with bipolar disorder had varying concentrations of several distinct metabolite species, depending on whether they reacted to lithium medication or not. The study's findings are consistent with the theory that BD is most likely brought on by the dynamic dysregulation of numerous proteins, metabolic pathways, and gene regulatory networks, which is an indication of intricate issues within the system.

Keywords: *BP; DSM; WHO; CNV; ANOVA.*

Introduction: Bipolar affective disorder, formerly known as manic-depressive sickness, is a neuropsychiatric ailment that is presently classified as a mood disorder. This diagnostic term for a medical disorder refers [1]to patterns of abnormal and excessive mood swings, ranging from periods of melancholy to heightened mood, or mania. Bipolar disorder (BD) manifests itself in a multitude of ways [1]. The effectiveness of treatment depends on the underlying disease and may coexist with other mental health issues. If left untreated, BD can have a terrible impact on a person's quality of life, as well as that of their loved ones and the community at large [2]. In the most severe cases of BD, there can be manic or depressive psychosis along with behavioral and cognitive problems. From the standpoint of public health, the condition is particularly problematic since it increases the death rate from suicide and necessitates repeated hospital stays [3].

Nosology, Symptoms, and Clinical Course of BD:
The spectrum of psychiatric disorders that are included in the term "bipolar disorder" is rather broad. In the late 20th century, clinical trials that followed patients over time found a startling variety of morphologies among the affected population. The bipolar spectrum includes mania without depression, dysthymia, cyclothymia, and mania types I and II. Some of the most prevalent kinds of mood disorders are shown in Figure 1, along with the symptoms that go along

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with them [4]. Being a chronic disorder, BD can worsen in several ways over the course of a person's life. A person with bipolar disorder (BD) may first experience depressive symptoms, manic or hypomanic symptoms, or a combination of these. Men are more likely than women to experience a manic first episode of bipolar disorder, but both sexes are equally likely to experience a depressed first episode [5]. If BD is not treated, a patient may experience more than ten emotional episodes in their lifetime. A typical 'euthymic' period occurs for most bipolar illness sufferers between manic and depressed episodes, during which they return to a roughly normal mental state. The length of manic and depressive episodes, as well as euthymia (periods of remission), varies greatly in individuals with bipolar illness. Compared to experiences of sadness or mania, manic episodes typically last less time [6].

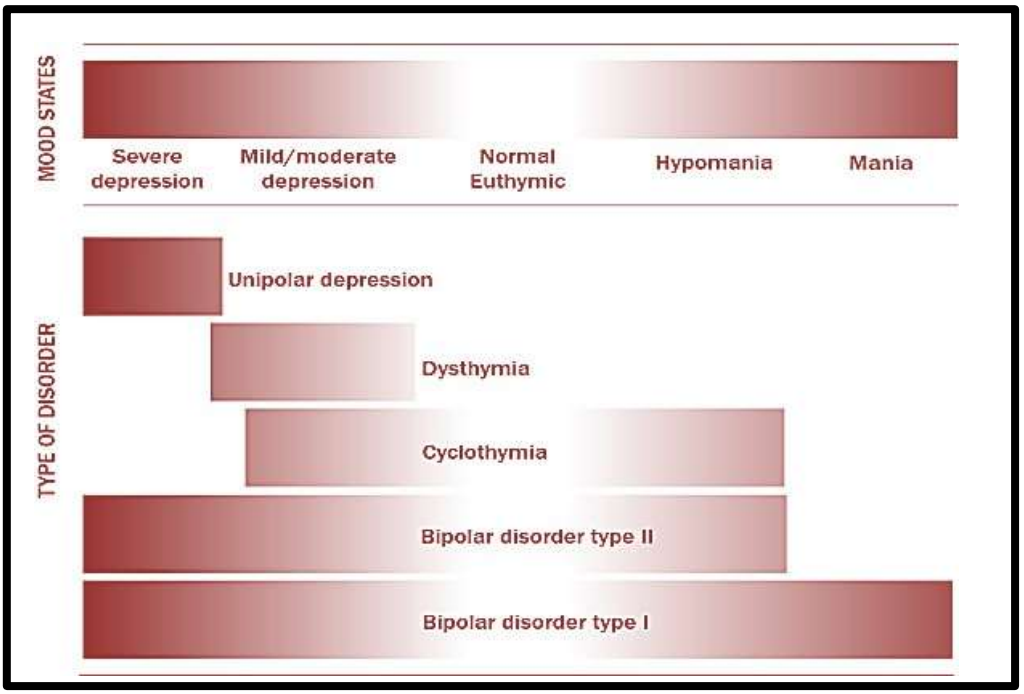

Figure 1: Characteristics of mood states in different types of mood disorders.

An average manic episode lasts, according to estimates, six weeks. A major depressive episode typically lasts eleven weeks, while a mixed episode lasts seventeen weeks on average. Compared to the first three seasons, the interval between episodes gets shorter in the latter seasons. The number of episodes and their duration start to progressively decrease and increase after the first three [7]. Patients with more than four episodes of mood disturbance annually are referred to be rapid cycling patients. Fast cycling is a common occurrence in adult females with BD (affecting 5-20% of them). While some BD sufferers may not exhibit any pattern of recurrent episodes, others do exhibit regular, predictable transitions from depression to mania or mania to depression [8]. A recent assessment evaluated the long-term consequences of BD-I in a community of South Indians. In 85% of cases, mania is the most prevalent and frequently the first sign of bipolar illness [9]. On average, 11% of patient lifetimes were accounted for by affective episodes, most commonly mania. The mean duration of a manic or depressive episode was two months, and the mean interval between relapses was 21 months [10–11]. Similarly, research in a rural Indian community and a cohort from northeast India discovered that BD frequently showed up as recurrent manic episodes.

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 behavioral 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 behavioral 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 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].

Genetics:

Although several distinct clinical findings about BD have been reported, the most persistent finding is that BD is inherited via families. Because of this, a significant percentage of ongoing research focuses on identifying risk factors for the illness, such as particular genes or chromosomal regions [13]. Even if the exact genes or chromosomal regions underlying BD are still unknown, genetic research in the twenty-first century has greatly advanced our understanding of the condition's biology. Genetic predispositions to BD have been linked to several physiological mechanisms as well as changes in the structure and function of the central nervous system. A variable illness history, late age of onset, parent-of-origin effects (POEs), and discordance in monozygotic twins are further characteristics of BD that are associated with epigenetic dysregulation [14]. Recent studies on BD have centered on epigenetic mechanisms. Further details regarding the genetics and epigenetics of BD can be found in the literature review included in this thesis [15].

Related work:

The past few decades have seen a great deal of research on adoptees, twins, and families, which has been essential in clarifying the heritable nature of BD. Based on family research, it has been estimated that first-degree relatives had a 10% relative risk of breast cancer, which is ten times higher than the general population [16]. First-degree relatives had a two-fold higher risk of unipolar depression. Unipolar depression has a high population prevalence and is more common in families where bipolar disorder has been present in the past. In addition, higher rates of schizophrenia and schizoaffective disorder have been discovered in bipolar families. Since different families have distinct histories, it is challenging to draw broad conclusions regarding how BD is transmitted. The presence of other psychiatric disorders in families with BD patients further obscures the true nature of heredity [17].

For dizygotic twins, the risk is 15–25%; if one twin has BD, there is a 70–85% probability that the other will as well. Considering these variances, BD has one of the greatest estimated heritabilities (70–85%) of any psychiatric disorders. Therefore, some of the risk (15–20%) is attributable to environmental influences, such as psychological problems, indicating that it is not solely inherited [18]. Compared to their biological families, adoptive families often have fewer members who suffer from BD, according to research on adopted individuals with the condition. These results add to the evidence that BD is a mental illness with a hereditary predisposition.

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. Evidence for 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 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].

Psychotic Symptoms:

Delusions and hallucinations are examples of psychotic symptoms of schizophrenia that some BD patients also experience. Mania or depression may coexist with the psychotic episode. More than half of BD patients will experience a psychotic episode at some point during their disease. Grandiose delusions are the most common symptom of psychosis, but there are other indicators as well, such as hallucinations, erratic emotions, and cognitive instability [29]. Individuals with BD who also experience psychotic symptoms might represent a unique subtype of the illness, one that is genetically vulnerable to schizophrenia and has similar etiological foundations. Numerous investigations have shown that psychotic traits tend to cluster in BD families, which has led to an increased interest in the genetic research of psychotic BD [30]. Linkage studies have connected several chromosomal regions to putative genes linked to psychotic BD risk. Studies on potential gene associations have connected COMT to this illness.

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.

Genomic Structural Variations:

For the past few decades, population and medical geneticists have devoted a great deal of attention to finding and mapping variations in the human genome. The majority of human genomic variability is composed of short genetic variations and structural variants [34]. The great bulk of the human genome is composed of short-range genetic variants, such as point mutations and common polymorphisms. Short tandem repeats, or STRs, are another type of repeat polymorphism that falls under this group. Shorter insertions and deletions are also included.

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 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.

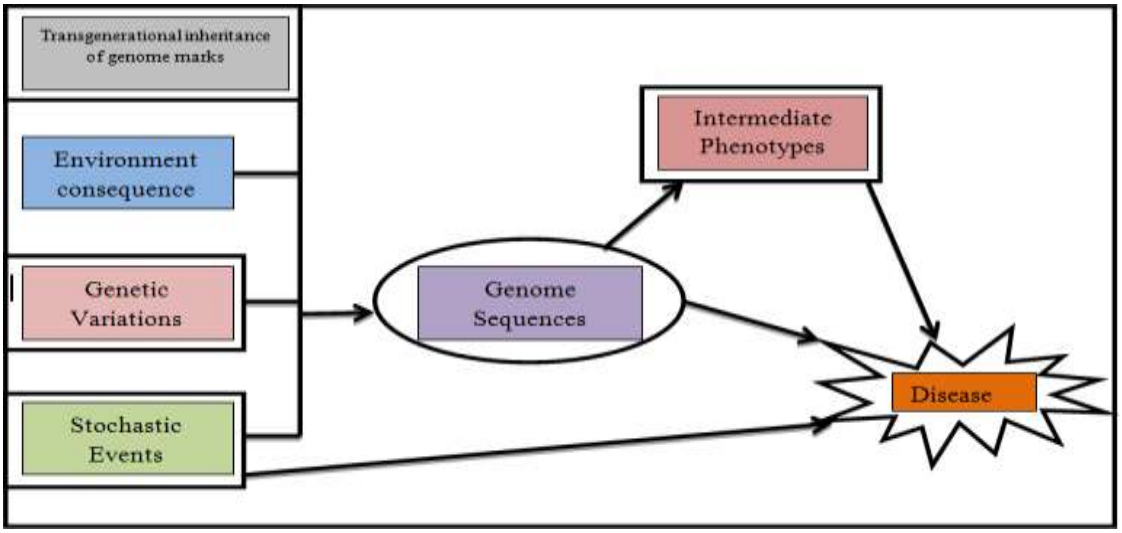

Figure 2: Inter-relationships among the epigenetic modification and other causal factors in complex psychiatric disorders.

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.

The objective of this research:

The purpose of this study was to help scientists better understand how various genetic events contribute to the phenotype of lithium receptivity in individuals with BD. This was carried out with the assumption that, as is possible in complicated disorders such as BD, evidence for further investigation could be found in the integration of multiple genetic anomalies. Consequently, the ensuing are our specific objectives. Using a genome-wide aCGH approach,

it is possible to find and confirm genomic areas and genes that have copy number variation (CNVs) in people with BD and to establish a correlation between these CNVs and the clinical subtypes and lithium response phenotype. examination of the plasma metabolites in a cohort of patients with bipolar disorder based on how responsive they were to lithium.

Proposed Algorithm:

The proposed flow diagram is shown below in figure 3.

Bipolar Disorder cases:

Following a comprehensive mental health assessment, a review of hospital outpatient department case files, and an in-person interview, BD was diagnosed based on the DSM IV-TR criteria (boxes 1, 2, 3, and 4). Each patient was assessed by the psychiatrists, who also provided diagnoses. In particular, the patients were first evaluated by a young psychiatrist who also painstakingly documented their clinical and mental histories. The final evaluation and treatment plan were overseen by a well-known psychiatrist. The selection of individuals was based on the fact that they were diagnosed with borderline disorder (BD), which was the primary cause of their significant discomfort or dysfunction and the reason they sought therapy. Ninety-five percent of BD patients were diagnosed as type I BD. Due to their association with cognitive impairment, head trauma, significant physical or neurological illnesses, substance use disorders, and mental retardation were excluded but not included. Patients having multiple Axis-I disease diagnoses were not included in our analysis.

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.

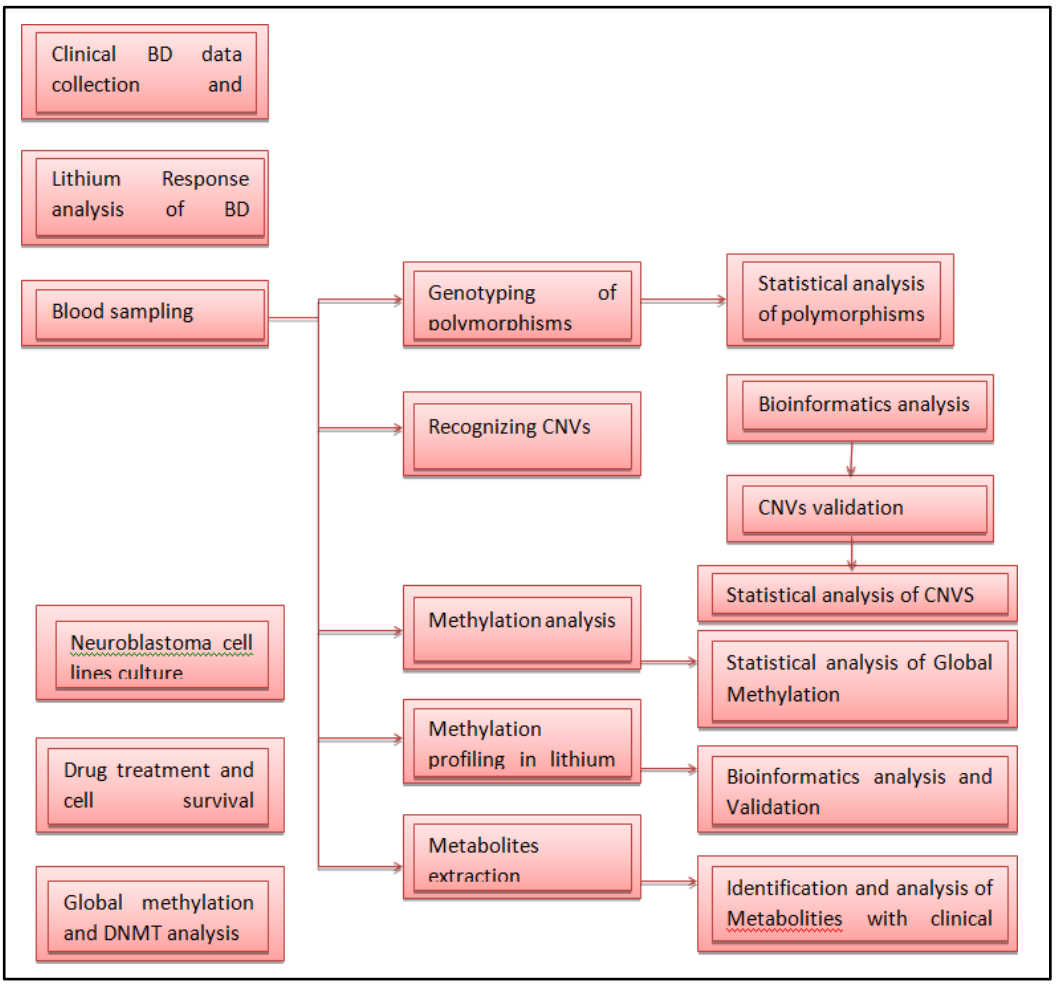

Figure 3: The proposed flow- diagram.

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, and 100 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 101bp (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.

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.

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.

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-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.

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.

Result and Discussion:

Of the 203 BD patients enrolled in the current trial, 63 were identified as lithium responders based on the response criteria. A single, sizable mental health facility served as the source of clinical subjects, guaranteeing consistency in the diagnostic processes and assessment of lithium response. Thirty-one patients could not be assigned to a different response group because they did not meet all inclusion criteria. Due to poor tolerance, side effects, intervening contraindications, poor therapeutic adherence to lithium, and insufficient information regarding treatment outcome, these patients were excluded from consideration for the assessment of lithium response.

In order to evaluate the potential impact of demographic and clinical differences between the two groups on the participants' lithium response outcomes in BD, we compared them. Frequencies and percentages were used to describe the clinical and demographic information of patients with bipolar disorder, lithium responders, non-responders, and controls. A Fisher's exact test was conducted to ascertain whether there was a statistically significant link between the categorical variables, whereas t rest was utilized for the continuous data. The patients experienced three bouts of aberrant mood and had a mean age of 14 years when they were first diagnosed with the condition. Manic episodes were the most prevalent among these patients and accounted for the great majority of hospital admissions. Both the frequency of episodes and the prevalence of psychotic characteristics were greater in lithium non-responders than in responders (mean difference = 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).

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

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

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

$$P - value = 10.\log \frac{Max}{i} \quad (2)$$

$$10\ \sqrt{MSE}$$

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 of BD	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		

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.

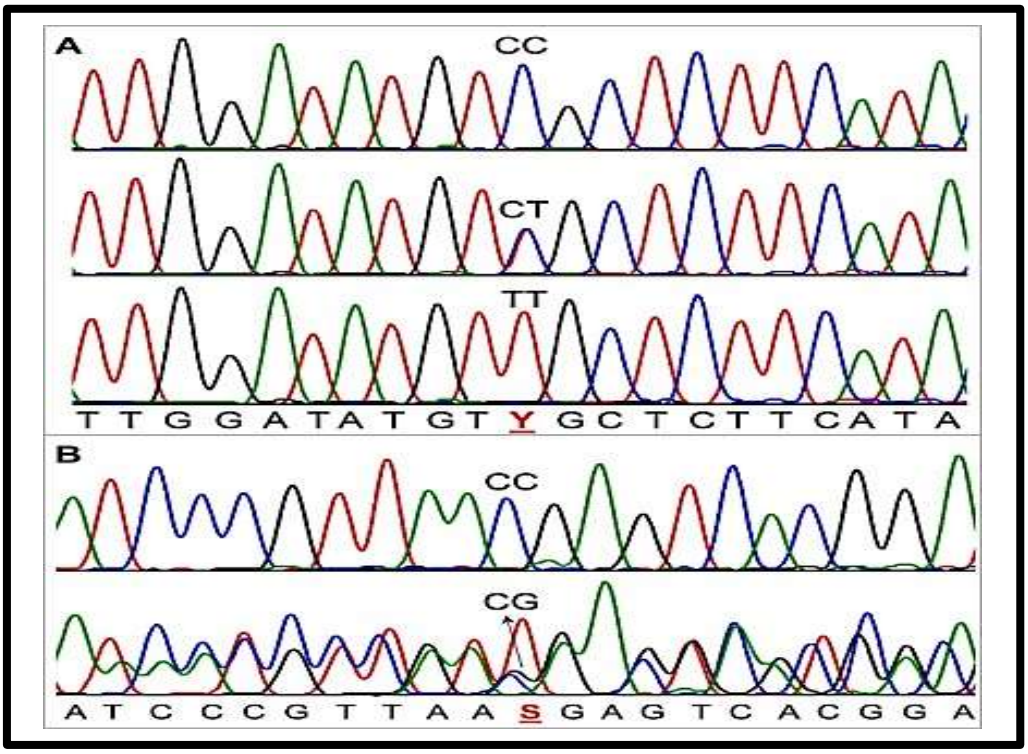

Figure 4: Sanger sequencing electropherograms of samples representing different genotypes.

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).

Table 2: CNVRs Count identified in the aCGH analysis

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

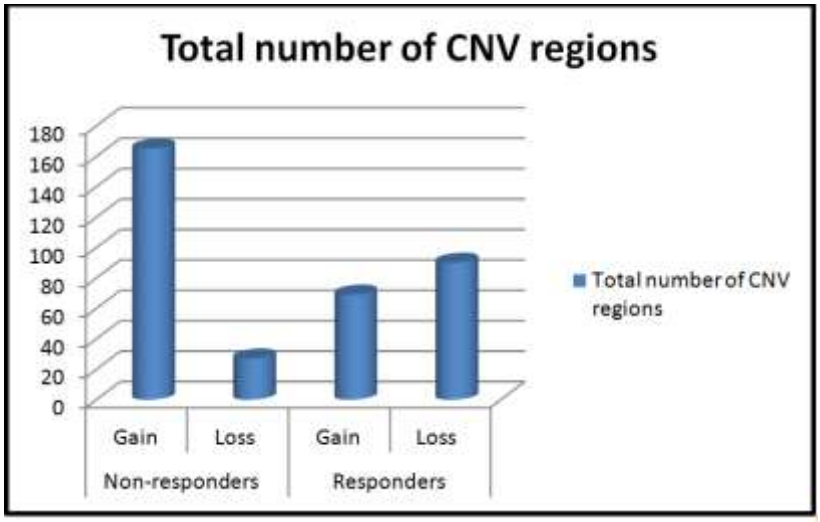

Figure 5: Total number of CNV regions for Responder and Non-responder type CNVs.

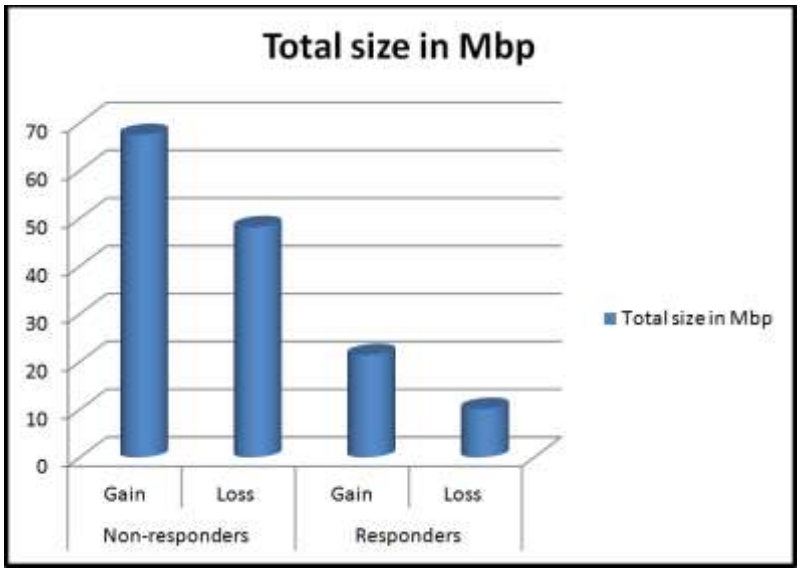

Figure 6: Total size in Mbp for Responder and Non-responder type CNVs.

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-responder's vs growth in responders. We did not, however, uncover any CNVs of this common but opposite type.

Conclusion and Future Scope:

Conclusion:

Neuropsychiatric disorders such as BD are thought to arise from dynamic dysregulation of several gene regulatory pathways, proteins, and metabolic networks, indicating intricate perturbations of the system. This study used a variety of techniques to identify molecular markers in an Indian population of bipolar disorder patients that were associated with the disease, lithium

responsiveness, and other subclinical characteristics. Retrospective classification of the lithium drug responses in patients diagnosed with main BD resulted in 62 responders and 103 non-responders. A group of subclinical signs, such as the existence of psychosis, a family history of mental illnesses, suicidal thoughts or actions, etc., were used to categorize subjects with BD. The rate of suicide conduct was significantly greater in lithium non-responders compared to responders when comparing clinical characteristics. Numerous investigations have demonstrated the extensive distribution of CNVs across the human genome, suggesting a major role for them in the vulnerability to neuropsychiatric illnesses. There is no proof that BD patients have a higher burden of CNV than healthy controls, according to the majority of genome-wide CNV studies. Unlike BD, concurrent nucleotide variations (CNVs) might be a distinct genetic factor that determines a person's vulnerability to diseases that have a stronger neurodevelopmental component. Our analysis revealed that non-responders had a much greater frequency of CNVs larger than 1 Mb than did responders. According to our analysis of the average size and CNV distribution, responders have a lower CNV burden than lithium non-responders. This study supports the hypothesis that people with a more severe form of BD with deeper neurological and/or neurodevelopmental bases are those who do not respond to lithium.

Future Scope:

Although more investigation is required to determine the precise function of the molecular markers found, the results presented in this paper represent one of the first attempts to shed light on the possible involvement of CNVs and epigenetic variables in BD, its subclinical manifestations, and the lithium response. Studying the aetiology of psychiatric diseases is still a challenging research topic due to our poor understanding of the type of biological variation that may contribute to disease and the inheritance process at play in complicated illnesses. With high-throughput DNA sequencing technology constantly evolving, there is a great deal of promise for the identification of novel genetic variants associated with psychiatric diseases. Large-scale whole-genome sequencing is expected to have a major positive impact on the search for the genetic basis of mental illnesses.

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