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The Role of Epigenetic Mechanisms in Depression, Bipolar Disorder, and Anxiety

Tarini Misra
tarinimisra21@gmail.com, tarini.3057@bis.edu.in

ABSTRACT

For generations, factors affecting cellular development and health were categorised as genetic or environmental. Epigenetics is a field at the cutting edge of biological and health sciences that draws connections between both genetic and environmental factors. While it shows tremendous potential for application in disease pathogenesis, neuroscience, psychiatry, and more, it has gained significant attention only in the last few decades. This paper aims to organise existing knowledge across studies on epigenetics and its connection with depression, anxiety, and bipolar disorder. It focuses on synthesising diverse studies, drawing connections between different findings, and providing a comprehensive view on the topic. The methodology includes primary and secondary research papers and journal articles. Raw data and the qualitative analysis have been discussed. Both human and animal studies were also analysed as relevant and applicable. This review first provides background information on key foundational concepts – types of epigenetic mechanisms, environmental factors that cause epigenetic changes, and technology in epigenetic detection. Later, it focuses on three mood disorders – depression, bipolar disorder, and anxiety – which are some of the most prevalent psychiatric conditions in the population, to explain the role of epigenetics in the development and onset of each of these, as well as in the quest for treatments. The findings suggest that epigenetic modifications play a significant role in altering neural pathways associated with emotion regulation, cognition, and stress response, showing correlation to behavioural disorders. Epigenetic changes have been found to be reversible, in some cases, showing their potential for therapeutic intervention. Overall, this paper concludes that epigenetics research and emerging technologies in epigenetics provide a critical framework and potential for understanding how environmental experiences interact with human biology and physiology in the development of behavioural disorders.

INTRODUCTION

The field of epigenetics has gained significant attention and relevance in recent years, for its ability to give insight into complex human behaviour like stress-response, emotional regulation, addiction, and even resilience, and answer highly debated questions about nature versus nurture. Traditional genetics focuses on fixed DNA sequences, but epigenetics goes a step further to explain the interplay between environmental factors and genetics, making it an essential area of study in contemporary neuroscience and psychiatry.[1]

Epigenetics is the study of heritable changes in gene expression that occur due to chemical modifications of DNA without a change in the underlying sequence of bases in the genetic code. It includes several mechanisms such as DNA methylation, histone modifications, non-coding RNAs, and chromatin remodeling, which will be discussed in this paper. These modifications are highly responsive to environmental factors like early-life adversity and trauma, exposure to stress, environmental pollutants, and lifestyle choices, all which have lasting effects on neural pathways.[2,3]

In recent years, epigenetics has been a critical area of study in the understanding of mental health and psychiatric disorders, and in developing treatments for these complex conditions. It has been revolutionary in providing evidence that draws the link between environmental factors and genetic predisposition to these disorders, which has long been suggested, but can now be far better explained through the study of epigenetics.[4]

Anxiety, depression, and bipolar disorder are three of the most globally common behavioural disorders today, and their prevalence continues to grow. These conditions also demonstrate strong influence of both genetic and environmental factors, making them key subjects for epigenetic studies.

The paper aims to provide a thorough and clear understanding of epigenetics and its role in these disorders, encompassing various perspectives from existing literature, exploring the limitations of these studies, and proposing topics for future study and practical application.

METHODS

Databases: This literature review was conducted using search engines such as Google Scholar and PubMed to provide a comprehensive study of relevant academic papers. Early on, the process involved the use of keywords like ‘epigenetics’, ‘behavioral disorders’, ‘mood disorders’, ‘DNA methylation’, ‘histone modifications’, ‘non-coding RNAs’ and ‘chromatin remodelling’. Keywords like ‘environment’, ‘lifestyle’, ‘pollutants’, and ‘cultural transmission’ were used to find information about the environmental factors in epigenetics, and later in the process, the keywords ‘depression’, ‘bipolar disorder’, and ‘anxiety’ were used to find studies for specific sections. The keywords mentioned were used with Boolean operators (AND, OR etc.) to find more specific results that were relevant to different parts of the paper.

The information about the mood disorders discussed was drawn from resources such as the Diagnostic and Statistical Manual of Mental Health Disorders, Fifth Edition (DSM-5), which is a globally recognized guide to mental health and neurological disorders, as well as data published by the World Health Organisation (WHO).

Time Range: Studies and articles published in the last 1-2 decades were preferred. All core primary and experimental studies on epigenetic mechanisms and their role in the discussed disorders were published

during a fifteen-year period 2009 and 2024. Sources on strengths and limitations of technology in epigenetics during different periods are from post 1995 according to the period of use of the technology.

Number of Studies: The findings from 30 studies have been synthesized and analysed in the Results and Discussion section, with additional sources serving as background information into concepts discussed.

Rationale for Study and Source Selection: The academic context for this paper integrates both literature reviews and primary scientific articles. The literature reviews provide a range of perspectives on the topic, as well as knowledge on the gaps that currently exist, both of which are important as it is still an evolving area of study. Additionally, qualitative data from primary articles was used to summarise evidence from previously conducted studies, provide raw data that can be analysed to draw connections, and understand areas for further research.

Peer-reviewed journal articles and systematic reviews were utilised, and overall, sources were selected based on relevance, credibility, and depth of academic context. Studies conducted on human subjects were favoured; however, animal studies were also used where relevant and applicable to humans, considering human studies in some cases are few due to the recent and complex nature of the study of epigenetics. Additional sources were also identified from the reference lists of key papers to obtain raw data or information in greater depth.

TYPES OF EPIGENETIC MECHANISMS

3.1 DNA Methylation

DNA methylation is one of the most studied types of epigenetic modifications, and refers to the addition of a methyl group (-CH2) to the DNA, typically causing inactivation of genes.[2,3] This binding usually occurs at the fifth carbon atom of the cytosine of the CpG dinucleotide.[2,5] The process of methylation is catalysed by enzymes called DNA methyltransferases (DNMTs), which are present in both prokaryotic and eukaryotic organisms. There are three main DNMTs - Dnmt1, Dnmt3a, and Dnmt3b. Dnmt3a and Dnmt3b are called the *de novo* (latin for ‘anew’ or ‘from the beginning’) transferases as they have the ability to methylate naked DNA, changing the way it is expressed. The methylation of DNA is typically associated with reduced gene expression. Dnmt 1, on the other hand, is known as the maintenance Dnmt as it conserves the original DNA by copying the exact pattern of methylation after DNA replication takes place.[2,3,5,6]

3.2 Histone Modifications

Histone proteins form the core of a nucleosome, the basic unit of chromatin. The DNA is wrapped around an octamer, or group of eight histones, comprising two each of the H2A, H2B, H3, and H4 histones. Histone modification refers to the different processes in which the histone tail undergoes post-translational changes, causing the DNA to wrap around the histones either more tightly or loosely. These processes include acetylation, methylation, phosphorylation, ubiquitination, and more.[2,3,7]

Acetylation, which is controlled by histone acetyl transferases (HATs), involves the addition of an acetyl group (-COCH3) to the amino acid lysine on the histone tail.[7] This increases the accessibility of RNA Polymerase to the genes, thus increasing gene transcription and expression.[8] The reverse process, deacetylation, is controlled by enzyme deacetylases (HDACs), which remove acetyl groups and promote chromatin condensation.[2,3,6] The process of histone methylation, which is controlled by histone methyltransferases (HMTs) silences the genes through the addition of a methyl group that causes the chromatin to condense, preventing transcription machinery from accessing the DNA.[3]

3.3 Non-coding RNAs

Non-coding RNA (ncRNA) are RNA molecules that do not encode for functional proteins yet play crucial roles in gene regulation. Only around 1% of DNA consists of protein coding genes, while 99% does not code for any proteins.[9] Although the role of ncRNAs has only recently been discovered, research has shown that many regulatory ncRNAs, including small interfering RNAs (siRNAs), microRNAs (miRNAs), long ncRNAs (lncRNAs) and more, have important functions in epigenetic control.[10,11] Long RNAs (typically 200 or more base pairs) are often highly cell specific and have diverse functions, from activating to silencing genes by modifying the stability and translation of mRNA, regulating chromatin function, and interfering with cell signalling. Other types of regulatory RNA like siRNAs and miRNAs are also involved in silencing genes and play a role in RNA interference to protect the body from foreign RNA.[11]

3.4 Chromatin Remodelling

Chromatin remodelling refers to the changes in chromatin structure that regulate DNA accessibility, and hence gene expression. Chromatin can be of two types, depending on its temporary structure and accessibility: euchromatin, or the open form, that allows for gene expression, and heterochromatin, or the closed form, that restricts gene expression.[3] This can be due to chemical changes, as discussed above, or the repositioning of nucleosomes. This is facilitated by several different enzymes like switching-defective/ sucrose-non-fermenting (SWI/SNF) enzymes, imitation-switch (ISWI) enzymes, chromodomain helicase DNA-binding (CHD) enzymes, and inositol-requiring 80 (INO80) enzymes.[12,13] Together they are involved in the spacing, assembly, sliding, and eviction of nucleosomes and determine overall chromatin organisation. This structure is responsible for facilitating access to genes, and regulates gene expression.[14] Misregulation of the nucleosomes can also lead to developmental defects and cancers, which is why understanding chromatin remodelling is considered an important step in progressing chromatin-based therapies.[12,14]

ENVIRONMENTAL FACTORS THAT CAUSE EPIGENETIC CHANGES

4.1 Environmental Pollutants

The exposure to environmental pollutants has been linked to epigenetic changes which can lead to the development of respiratory and other kinds of diseases like cancer and cardiovascular disease.[15] Pollutants including particulate matter (PM) such as adsorbed metals and black carbon, and harmful gases such as sulphur dioxide and nitrogen oxides have been shown to decrease levels of DNA methylation, thus

modulating gene expression. These changes have been seen to occur due to exposure in utero, as well as in childhood and adulthood, with the effects of in utero exposure also lasting into later life. Studies have also been conducted on the role of duration of exposure and concentration of the pollutants. These studies have shown that the higher the concentration, the greater the effect on methylation patterns. Similarly, longer exposure also caused increased changes to methylation patterns.[16]

4.2 Alcohol Consumption

Long-term alcohol consumption causes a decrease in the levels of S-adenosylmethionine, a natural compound found in the body tissues that plays an important role in cell functions.[17] S-adenosylmethionine is a key methyl donor which provides a substrate for DNA methyltransferases and histone methyltransferases for DNA and histone methylation, respectively. Thus, a decrease in S-adenosylmethionine levels causes hypomethylation, or lower levels of methylation than usual.[17,18] Furthermore, regular histone acetylation patterns are affected by alcohol consumption, due to the increase in the ratio of reduced nicotinamide adenine dinucleotide (NADH) as compared to oxidised nicotinamide adenine dinucleotide (NAD+), which is associated with issues like accelerated aging and mitochondrial dysfunction.[17,19,20]

4.3 Psychological Stress

Studies into the effects of both chronic, or long-term, and acute, or short-term, stress on epigenetic mechanisms have yielded diverse results, suggesting a complex system of factors involved. Exposure to stressors causes the production of steroid hormones known as glucocorticoids, whose effects are regulated by the hypothalamic-pituitary-adrenal (HPA) axis.[21] A study conducted on mice at the Johns Hopkins University School of Medicine showed that increased exposure to corticosterone, the primary stress hormone produced by mice, altered the expression of three HPA axis genes and decreased methylation of *Fkbp5*, which produces FKBP5, a protein that modulates intracellular glucocorticoid signalling.[22,23] Genetic modifications to *Fkbp5* have also shown correlation with the development of mood disorders and post traumatic stress disorder.[22] Overall, stress-induced epigenetic modifications affect gene expression in the brain, thereby changing future stress responses.

4.4 Cultural Inheritance

Cultural inheritance is defined as 'the storage and transmission of information by communication, imitation, teaching and learning'.[24] Cultural influences, including childhood environment, race and ethnic background, and socio-economic status, have been seen to have strong effects on human epigenetic marks such as methylation. A study conducted at Northwestern University established the socio-economic status of 489 participants based on income, assets, and education, and aimed to determine the impact of this on the epigenome, observing changes in methylation patterns at CpG sites. They found significant effects on over 2500 sites, with the methylation of 1777 sites increasing and 769 decreasing due to low socio-economic status.[25] Parental traits–including personality, intelligence and mental health–as well as the behaviours elicited due to these traits have been shown to affect the child's epigenome. This can subsequently affect a child's personality, behavior, cognitive abilities and much more.[26] Cultural factors in epigenetics are extremely hard to study due to the significant variance present in any environment, making it difficult to obtain consistent results.

ROLE OF TECHNOLOGY IN EPIGENETIC DETECTION

Bisulphite sequencing, invented in 1992, was considered the foremost method for detecting DNA methylation, differentiating unmethylated cytosine and 5-methylcytosine on the basis of amination patterns.[27] Post treatment with sodium bisulphite, cytosine is converted to uracil residues, while 5-methylcytosine remains unchanged, allowing for its identification during subsequent PCR steps.[28] However, this method, while still prevalent today, causes significant degradation of DNA due to depyrimidination, resulting in decreased volumes of full-length DNA in PCR quantification.[29]

With the significant advancements in next-generation sequencing (NGS), specifically the introduction of second-generation sequencing, since the early 21st century, epigenetic detection has moved rapidly towards sequencing-based methods. A key technique in NGS for epigenetic detections is Illumina, which utilises sequencing-by-synthesis (SBS).[30] To overcome the challenges of bisulphite sequencing, enzymatic methyl-seq (EM-seq) was discovered.[31] Two enzymes, TET2 and T4-BGT, convert the methylated 5mC and 5hmC into products which cannot undergo deamination by APOBEC3A.[32] Therefore, only the unmodified cytosines are deaminated to form uracil, which is read as thymine, and can be distinguished post amplification when the DNA is sequenced using Illumina.[33]

The short-read market is still roughly 11 times larger than the long-read market[34,35], with Illumina offering the benefit of extremely high accuracy due to clonal amplification, or clustering.[36] Approximately 1000 amplified copies of the same sequence are synthesised base-by-base, each generating fluorescence, thereby reducing the impact of a few anomalies within the cluster and increasing overall accuracy in sequencing.[37]

On the other hand, long-read sequencing, or third-generation sequencing, such as single-molecule real-time (SMRT) sequencing by Pacific Biosciences (PacBio) and Nanopore sequencing by Oxford Nanopore Technologies have become increasingly popular in the last decade.[38] They offer the additional advantages of detecting structural variations, assembling complete genomes, and sequencing regions with tandem repeats, thus providing a more holistic genome analysis.[39] Furthermore, nanopores use non-amplified, native DNA, which allows for real-time observation of epigenetic modification patterns on the original DNA, and also eliminates the need for a separate library preparation for epigenetic studies.[40] SMRT makes use of sequencing-by-synthesis, with fluorescent-tagged nucleotides forming a complementary DNA strand. As each nucleotide is incorporated, the cluster generates a fluorescent 'pulse', and the interpulse duration (IPD) can give insights into methylation patterns.[41] The kinetic variation is seen due to the lower speed of DNA polymerase when incorporating methylated nucleotides, resulting in greater IPD and helping identify methylation within the sequence.[42]

Nanopore devices include flow cells consisting of an electro-resistent membrane with an array of tiny holes, each connected to an electrode.[43] As nucleic acid molecules pass through the nanopore, the electric current is disrupted, as detected by a sensor chip. Due to the different chemical properties of the bases - A, T, C, and G - they create differing 'squiggles' of data, which are decoded by real-time basecalling

algorithms.[43] The electric current signals also differ for methylated and unmethylated nucleotides, helping distinguish between them.[44]

Despite being some of the most advanced methods, SMRT and nanopore have their limitations when it comes to observing epigenetic modifications. SMRT requires minimum 250x coverage per strand for 5-methylcytosine detection.[44] While the error rates for nanopore have significantly reduced with technological advances in recent years, they can still be as high as 10%.[45] Additionally, nanopore methods require vast and complicated training data sets from confirmed methylation samples, as well as complex and sophisticated computational algorithms.[46]

Overall, the consistent development in technologies that detect epigenetic modifications has allowed great progress in the field and the applications of epigenetics research. Greater innovation to improve long-read techniques, decrease DNA degradation, and reduce error rates can enhance this further. Furthermore, the development of techniques, beyond those that currently exist, to detect epigenetic changes apart from methylation would also be beneficial, as evidence highlighting the significant roles of histone modifications, chromatin remodelling, and non-coding RNAs is becoming more and more prevalent.[47]

EPIGENETICS AND DEPRESSION

Depression, formally known as depressive disorder, is one of the most commonly occurring mental health disorders, experienced by an estimated 3.8% of the population, according to the WHO. It is also predicted to become the greatest disease burden by 2030 in terms of financial cost, morbidity, and mortality.[4,48] Depression is approximately 50% more common in women than in men and is estimated to occur among 3.5% of adolescents aged 15 to 19 years.[49]

Due to the significant interpersonal variation in the causes of depression, it is extremely hard to establish direct causal relationships in any given case.[48,50,51] Despite the existence of effective methods of treatment today, over 75% of the population in low to middle income countries does not receive treatment for depression due to lack of access to mental health care and social stigma around mental health conditions.[49] Depression is characterised by consistent depressive mood and a loss of interest and pleasure. Symptoms range from weight fluctuation, fatigue, reduced thought and mental capacity, and excessive feelings of worthlessness and guilt, to recurring thoughts of death, as outlined in the Diagnostic and Statistical Manual of Mental Health Disorders, Fifth Edition (DSM 5).[49,52,53]

Evidence from epidemiological studies suggests that depression is potentiated by a combination of genetic and environmental factors, such as stressful life events, personality traits, pregnancy, menopause, loneliness, alcohol and drug consumption, or long-term illness.[54]

When it comes to genetics, DNA methylation is the most studied epigenetic process in the development of Major Depressive Disorder (MDD), a chronic form of depression that is diagnosed as a subset of depressive disorder. Increased or decreased methylation patterns have been observed in different genes

that are responsible for modulating neurotransmission in the brain and regulating mood, both of which are significant factors in the progression of depressive disorder.[48,51,55–57]

Research on histone modifications in depression has also been conducted but primarily focussed on histone methylation and acetylation. Increased acetylation has been observed in the nucleus accumbens (NA), also known as the pleasure center, in post-mortem studies of patients with MDD. Similarly, increased histone methylation has been observed in the prefrontal cortex of MDD patients.[51,58–61]

Certain non-coding RNAs, namely miRNAs, have also been studied in conjunction with MDD. Altered levels of miRNAs have been detected in patients with MDD and other psychiatric conditions. miRNA regulates gene expression by binding to specific sites on the mRNA in the post-transcriptional phase, thus affecting the activation of some genes that are involved in the progression of disease.[50,62–64]

Due to the growing occurrence of depression in the population, and the initial indications that epigenetic marks may have a role to play, many of these mechanisms and their role in the onset of depression have been highly studied.

EPIGENETICS AND BIPOLAR DISORDER

Bipolar disorder (BD) is a mental health condition faced by approximately 1 in every 150 adults or an estimated 40 million people, including individuals in adulthood as well as the youth, according to the WHO.[65] The occurrence is roughly equal in men and women, but early onset has been more commonly observed in males, while attempted or completed suicide due to the disorder has been more frequently observed in females.[65,66]

The treatments for bipolar disorder have been developed to include a combination of medicines and drugs along with psychological and psychosocial interventions. However, in many cases, even arriving at the stage of treatment is inhibited by misdiagnosis and lack of resources and funding for BD treatment, especially in low-to-mid income countries.[65] Lack of knowledge about the condition and the difficulty in managing it due to its unpredictability and fluctuating nature have built up a social stigma and led to those suffering being neglected in society.[67]

Bipolar disorder is defined as extreme fluctuations in a person's mood, energy, and ability to engage in daily activities due to oscillating periods of mania (or euphoria) and depression (or hopelessness), according to the DSM 5.[68] It can impact concentration, create excessive feelings of guilt, cause loss in appetite and sleep, exhaustion, and even suicidal behavior.[65]

Genetics plays a significant role in bipolar disorder, with much higher prevalence in individuals with a first degree relative, such as a parent or sibling, suffering from the condition. It is also affected by periods of high stress, trauma, and drug or alcohol abuse.[69] Additionally, it is greatly influenced by the brain,

including the size of certain parts of the brain like the hippocampus, as well as imbalance in brain chemicals called neurotransmitters.[70]

DNA methylation in patients with bipolar disorder has been extensively studied, with observations including methylation of enzymes that control the levels of dopamine release.[4] Hypermethylation, which decreases gene expression, has also been observed in the brain-derived neurotrophic factor (*BDNF*) and the serotonin receptors in bipolar disorder patients.[71]

Histone modifications such as acetylation or deacetylation and trimethylation have shown some importance in the development of bipolar disorder. Increased acetylation of some HDACs and decreased acetylation of some has been observed. Trimethylation of synapsin genes, which modulate neurotransmission in the brain, has also been observed.[72]

The role of non-coding RNAs in bipolar disorders is relatively less studied and well-known. However, recent evidence has implicated lncRNAs, which bind to certain genes and regulate their expression. It must be noted that altered levels of lncRNAs have been observed in males, but no significant difference has been observed in females.[73]

The difficulty in finding specific genes that cause bipolar disorder is what led to the search for the epigenetic factors. Though relatively new, this is heavily studied and has also shown potential for use in treatments.

EPIGENETICS AND ANXIETY

Anxiety disorders are a group of mental health disorders that affect over 301 million individuals, or approximately 4% of the population, making them the most common mental disorders, according to the WHO.[74] Anxiety occurs twice as often in women as in men and is also the most prevalent emotional disorder in adolescents, particularly those aged 15 to 19.[75–77]
Treatments for anxiety disorder include psychological interventions such as talk therapy, as well as antidepressant medications and benzodiazepines, which can help slow down brain activity and regulate the nervous system.[74,78] However, despite being highly treatable, fewer than 37% of individuals with anxiety disorder receive treatment due to many reasons including lack of awareness, social stigma, and limited access to specialised services and trained professionals.[74,76]

Anxiety disorders cause severe dread, fear, and extreme reactions that are disproportionate to the circumstances that caused them. They can also result in physical manifestations like palpitations, shortness of breath, muscle tension, insomnia, and more.[79] There are 11 types of anxiety disorders defined in the DSM 5, of which the most common are Generalised Anxiety Disorder, Panic disorder, Phobia-related disorders, Agoraphobia, and Social Anxiety disorder.[80,81]

Anxiety disorders have been shown to result from a combination of social, psychological, and biological factors, as well as physical causes such as tension, hyperactivity of the nervous system, and alcohol use. They also develop more frequently in individuals who have experienced detrimental circumstances such as abuse or severe loss.[74] These environmental influences have been shown to cause epigenetic changes, through mechanisms such as DNA and histone modifications, as well as miRNAs, that have shown correlation with the development of anxiety disorders by modifying genome functioning.[82]

The most commonly observed DNA modification with regards to anxiety is DNA methylation. DNA methylation patterns have shown some differences in individuals with anxiety, in several genes that regulate stress responses as well as in regulation of the HPA axis.[82–84]

Histone modifications including methylation, acetylation and the other types discussed above have also been studied in conjunction with anxiety disorder, using rodent models to observe epigenetic changes. For example, increase methylation associated with glucocorticoid receptor promoters were observed in the amygdala and hippocampus.[85]

The critical role of miRNAs in central nervous system development and homeostasis have made them highly studied with regards to anxiety disorders. Correlations have been found between the expression of certain miRNAs and anxiety symptoms, even down to correlations with the specific types of anxiety disorders mentioned above.[86]

Given the prevalence of anxiety disorders and the success seen with epigenetic therapies in the treatment of mood disorders, researchers have conducted several studies to further unravel epigenetic mechanisms underlying such disorders. The findings of these studies are discussed below.

RESULTS

9.1 Depression

A recent evaluation of 67 studies conducted worldwide found significant hypermethylation in peripheral tissues of individuals with depression. The hypermethylation was seen on the *BDNF*, and in the *SLC6A4*, a major focus of depression studies due to its role in serotonin transport. Due to the role of methylation in reducing gene expression, decreased levels of BDNF were observed.[48,87]

On the other hand, some studies have found differing results. For example, pregnant mothers with depressive disorder, and their infants, showed reduced methylation in the *SLC6A4* gene. Meanwhile, in adolescents suffering with MDD, no changes in methylation patterns of *SLC6A4* were observed, when compared with controls of the same age that did not have the disorder.[48]

Since methylation is specific to the type of cell, it has also been studied in both the blood and port-mortem brain tissue to ascertain its impact on depression. Common changes in methylation patterns

have been observed in both the blood and the Brodmann area 10 (BA10), which is a region of the brain responsible for complex thought and decision-making. *BDNF* showed similar changes in genes in the blood as well as BA10. The same can be said for two other genes, *GABBR2*, which helps regulate inhibitory brain signals, and *RUFY3*, which supports neuron growth and connectivity. These genes in patients with MDD showed changes in the blood, BA10, and BA25, which is involved in the regulation of negative mood.[87,88]

The observation of methylation in different tissue types provides convergent evidence for association between methylation and MDD, suggesting its importance as an MDD marker. The variance in certain findings could suggest unexplored factors that may be genetic, environmental, or influenced by demographic characteristics. The study of brain tissue, however, provides high biological validity due to the examination of genetic marks directly in disorder-relevant regions, minimising the limitations of tissue-specificity in epigenetic modifications.

In a study conducted on the post-mortem nucleus accumbens of the human brain, HDAC2 was decreased, showing a correlation between the dysregulation in acetylation patterns and MDD. Further, in a study conducted on mice, suberanilohydroxamic acid, which is an HDAC inhibitor was induced directly into the NA, resulting in a decrease in depressive-like behaviours, further demonstrating the role of histone acetylation in depression.[60] The study of rodent models may not fully replicate effects in human depression but provide stronger evidence for direct association under controlled experimental conditions.

Another study into histone methylation found similar results in 18 MDD patients who died by suicide. Trimethylation of Histone H3 at Lysine 4 (H3K4me3) was observed at synapsin 1, which regulates neurotransmitter release, resulting in overexpression of synapsin 1a (SYN1a) and synapsin 1b (SYN1b) in all of them.[51,58] However, the small sample size and selective sampling of patients who died by suicide for this study limit the generalizability of the study to all patients with depression.
The role of miRNAs in MDD has been studied by examining peripheral blood tissue, cerebrospinal fluid, and post-mortem cortex in both human and animal subjects. Alteration of several miRNAs, namely, miR-330-3p, miR-345-3p, miR-425-3p, and miR-24-3p, was observed in peripheral blood DNA.[64] Additionally, in the prefrontal cortex (BA9), 21 miRNAs were seen to be downregulated, including miR-142-5p, miR-101, miR-137, and miR-301a.[63] Altered expression levels were also observed in the BA10, BA44, anterior cingulate cortex (ACC), and other brain regions.[50,62] miRNA dysregulation appears to represent a recurrent epigenetic feature of MDD, observed across multiple brain regions and peripheral tissues, but lack of convergent findings on implications of specific miRNAs limits consistency and applicability in defining epigenetic markers.

9.2 Bipolar Disorder

Hypermethylation of *BDNF* has been observed in peripheral blood DNA of bipolar disorder patients through two methods: Methylation-specific polymerase chain reaction and bisulfite sequencing. Several studies have found greater levels of methylation of *BDNF* in patients with BD II, which is characterised by less severe high (hypomania) but more common depressive episodes, as compared to BD I which causes more severe manias rather than depressive episodes.[71,72,89]

Also in the peripheral blood, methylation on the CpG sites of 5-HT3AR, a serotonin receptor, was observed. It drew the link between childhood maltreatment and severity of BD in adulthood. 5-HT3AR was also observed in the frontal lobe and several other samples like leukocytes and saliva.[71,72,90] The detection of 5-HT3AR methylation across peripheral and frontal lobe tissues suggests partial tissue concordance, wherein similar gene alterations in both solid and circulating liquid tissue occur. Though broader replication across brain regions may be limited, this indicates high biological validity of the more broad-spectrum study.

Increased methylation of *FKBP5* has been observed in peripheral blood tissue, leading to altered expression of the gene, which is involved in regular stress signalling pathways.[71,91] In monozygotic twins with discordant diagnosis for BD, hypermethylation of *SLC6A4* was observed in the twin with BD.[92] The observation of consistent methylation changes across multiple genes strengthens the overall evidence for epigenetic dysregulation in bipolar disorder. However, as the majority of these findings derive from peripheral tissues, their direct applicability to central nervous system processes is limited by tissue specificity in epigenetics.

As mentioned above, histone deacetylases inhibit gene accessibility, so measuring expression of HDACs in BD patients has given insight into the effect of deacetylation. In BD patients, HDAC4 mRNa showed increased expression, while HDAC6 and HDAC8 showed decreased expression during a depressive state, as compared to healthy controls.[59,72] Varying expression and acetylation patterns suggest that these mechanisms may be state-dependent, during depressive episodes, rather than fixed markers for the condition. Global acetylation, methylation, and phosphorylation of the H3 histone was also observed in post-mortem studies of BD patients.[93] This provides stronger evidence than single-gene findings, suggesting broader mechanism-based modifications, but still cannot provide direct causal linkage.
With regards to histone modifications, trimethylation of *H3K4* has been studied in post-mortem brain samples of BD patients. This process is thought to open up the chromatin and increase transcription.[94] Increased H3K4 trimethylation was observed in synapsin genes, namely SYN1, SYN2, and SYN3 of the patients.[72]

The effect of lncRNAs in bipolar disorder is a more recent area of study in which evidence is being found. In a study conducted on 50 BD patients and 50 control subjects, the levels of five lncRNAs, H19, SCAL1 (LUCAT1), RMST, MEG3 and MT1DP were evaluated. In BD patients, the levels of SCAL1, RMST and MEG3 were greatly decreased, most significantly in males, while levels of H19 and MT1DP showed no significant change.[95] Another study on peripheral blood cells measured expression levels of lincRNA-p21, lincRNA-ROR, and lincRNA-PINT in 50 BD patients as compared to 50 healthy individuals. Similar to the previous study, significant downregulation of these RNAs was observed in male subjects with BD.[96] Convergent observations in more than one cohort strengthen exploratory findings for the involvement of these non-coding RNAs, but small sample sizes and relatively recent studies indicates these associations are emerging rather than definitive.

9.3 Anxiety Disorder

A study aimed at observing methylation of the *NR3C1* gene, which helps in regulation of the HPA axis, was conducted using 48 pairs of monozygotic twins as subjects, including pairs where both, one of the two, or neither of the twins had a diagnosis.[84,97] DNA from blood samples was extracted and methylation at CpG sites on two parts of the gene, exon 1D and exon 1F, was observed. Hypermethylation at exon 1D was seen in individuals with family history of anxiety, while no significant methylation changes were observed on exon 1F.[84,98] The monozygotic twin study design controls for genetic influences, thereby providing strong correlative evidence for environmental and epigenetic factors, but its feasibility is limited by the availability of monozygotic twin samples, reducing sample size and statistical power.

Studies have also been conducted to determine the role of methylation of *SLC6A4* and *BDNF* in the development of anxiety. Greater BDNF methylation was observed in patients with anxiety compared to controls, in women aged 65 and above.[99] Changes in *SLC6A4* methylation were observed after Cognitive Behavior Treatment (CBT) in children with anxiety, yielding a result that demonstrated the role of *SLC6A4* in the disorder. Individuals who responded to the treatment showed increased percentages of DNA methylation while non-responders showed a decrease in methylation.[100]

Similar to the previous study, to determine the effect of methylation of *FKBP5*, change in methylation after Cognitive Behavior Treatment (CBT) was measured. Amongst those who responded to the treatment, decrease in *FKBP5* methylation was observed in individuals who had agoraphobia, or other specific phobias, as well as in children with anxiety disorder, which was studied in another experiment.[23,85] The pre-post treatment experimental method provides strong temporal evidence and supports the idea that stress-related epigenetic marks may remain modifiable rather than fixed, but does not directly reflect epigenetic marks that determine disorder presence alone.
MeCP2 is a gene that encodes for the protein MeCP2, which is important in maintaining synaptic connections between neurons. In a study conducted on mice, deletion of the *MeCP2* gene resulted in an increase in anxious behaviors. Simultaneously, acetylation of the H3 histone was observed.[85,101] Gene knockout models provide strong mechanistic evidence, helping establish stronger experimental causality within controlled settings.

Another study on early life stress rats measures the levels of HDAC1-3 in the amygdala, finding increased expression of HDAC1 and HDAC2. Conversely, HDAC3 showed no significant difference when compared to control subjects, showing the variable role of HDACs in anxiety.[102] Studies in anxiety-related rodent models suggest that H3K4me3 is altered in brain regions implicated in fear and stress response, but human evidence remains limited.[103] Despite strong experimental control, the external validity of rodent findings to human application may be limited.

In order to study the effect of miRNAs on anxiety development, levels of several different miRNAs in peripheral cells like blood, and in post-mortem brain studies, have been observed. Circulating miR-4505 and miR-663 were seen in patients with generalised anxiety disorder while expression of miR-29c showed an increase in patients with exposure to social anxiety.[86,104,105]

Port-mortem samples of patients suffering from anxiety along with depression have commonly been studied. In these studies, downregulation of miR-135a was observed in the raphe nuclei, which is a cluster of neurons located near the midline in the brain stem that contain serotonin.[86,106,107] Several variations that are associated with anxiety disorders like panic disorder were observed in miRNAs like miR-22, miR-138-2, miR-148a, miR-339, and miR-488.[86,108] Across peripheral blood and brain tissues, miRNA dysregulation emerges as a recurrent feature of anxiety disorders. The overlap of certain miRNAs with depressive phenotypes suggests partial epigenetic convergence. Nevertheless, the wide range of implicated miRNAs and variability across studies indicates limited consistency at the individual-marker level.

DISCUSSION AND CONCLUSION

The brain-derived neurotrophic factor has important roles in ensuring neuronal survival and in neurotransmitter modulation, and hence has been studied with regards to the disorders mentioned above. *BDNF* hypermethylation was observed in both depression and bipolar disorder. This is associated with reduced levels of BDNF, which have been observed during depressive episodes. Especially in BD II, which is characterised mainly by depressive episodes, higher methylation was observed. Taken together, this suggests an involvement of BDNF in mood regulation, specifically in the occurrence of depressive episodes, and also demonstrates certain similarities between symptoms in depression and bipolar disorder.

These findings could aid in the development of BDNF gene therapies and treatment for both depression and bipolar disorder. However, it could also lead to misdiagnosis because the symptoms observed in depressive disorders and certain cases of bipolar disorder can be extremely similar. The differing results of methylation in BD I and BD II also illustrate the case specific nature of disorders like this, which makes it difficult to define universal epigenetic markers.

Another similarity between depression and bipolar disorder was observed in acetylation, with patients of both disorders exhibiting changes in expression of HDACs. In Depression, decrease in expression of HDAC2 was observed, similar to the decrease in expression of HDAC6 and HDAC8 in bipolar disorder. In BD, however, HDAC4 showed increased expression, once again demonstrating the complexity of epigenetics in the study of mood disorders, as reasons for why some HDACs increase expression and some decrease expression has not yet been elucidated.

Hypermethylation of the gene for SLC6A4, a serotonin transporter, was observed in peripheral tissue of patients with depression. Since methylation is believed to reduce gene expression, it is typically associated with reduced serotonin, which is a characteristic of depression.

Similarly, reduced serotonin is observed in anxiety. However, the results for *SLC6A4* methylation in anxiety patients were somewhat contradictory, with increased methylation being observed in cases of successful CBT. The idea that anxiety symptoms were reduced despite a decrease in serotonin suggests a

more complex role of serotonin, and supports another line of studies that demonstrate how certain neural connections actually increase anxiety when serotonin is too high.

Furthermore, for depression, the results observed in pregnant women and adolescents differed from the rest. This could be a result of the genetic variance between humans, which is not as prevalent in rodent species, which are inbred. It also suggests the role of environmental factors that have not yet been determined.

The inconsistency in results suggests that these studies need to be expanded to include a wider range of subjects, across age, sex, and environmental conditions. This also brings out the lack of knowledge and study into the possible effect of hormones on epigenetic findings.

Despite the fact that gender based differences in the findings have already been noticed, there is a lack of gender-specific studies thus far. For example, in BD, the onset age is earlier in men, while suicide rates are higher in women. Additionally, levels of lncRNAs were altered in males with BD but not females, meaning that the changes in females are still unknown and further studies into this can be conducted. Furthermore, both depression and anxiety are twice as common in women as compared to men, but reasons behind this are still largely unknown. The differing results of *SLC6A4* methylation in pregnant women with depression also suggests that the role of sex hormones such as oestrogen and testosterone should be studied. There is scope for further research into the genetic and epigenetic reasoning that may exist behind these differences.

The downregulation of miRNAs in both depression and anxiety suggests that they have similar effects in both disorders. Since miRNAs usually suppress gene expression, the downregulation could be correlated with increased neurotransmission. This could, in turn, cause increased release of certain stress hormones or even serotonin.

In both BD and anxiety, hypermethylation of *FKBP5* was observed. This observation in BD along with anxiety could suggest the presence of some anxiety-like characteristics and symptoms in BD as well.

DNA methylation and histone modifications have been far more studied due to the relative ease in observation and availability of established methods of study. Meanwhile the nucleosome remodelling is much less clearly understood, and does not appear in many studies regarding the mood disorders discussed above.

Epigenetics markers are crucial in understanding the effect of gene regulation in disease, and in diagnosis and treatment. Several studies have been conducted on epigenetic modifications that show the potential for being used as biomarkers in mood disorders. For depression, factors related to the HPA axis, as well as neurotrophic factors like BDNF have been identified as useful biomarkers for diagnosis. Genes encoding proteins like FKBP5 and serotonin transporters like SLC6A4 could also be helpful biomarkers in depression.[109] While determining reliable biomarkers in bipolar disorder has been difficult, relating different biomarker findings could help in its diagnosis.[110] Further study into mechanisms other than DNA methylation is also necessary. In anxiety, epigenetic changes in genes related to regulation of the HPA axis, such as *NR3C1*, appear to be the most reliable biomarkers. Epigenetic changes in other genes like

BDNF, *FKBP5*, and *SLC6A4* have also been most commonly observed in conjunction with stress experiences, making them promising markers.[82]

Epigenetic marks are extremely cell-specific, meaning that the epigenetic modifications could greatly differ between cell types. This creates a limitation in the studies due to the fact that many of them have been performed on peripheral tissue such as blood cells. In cases where both peripheral tissue and brain cells were studied, the epigenetic marks often did not exactly match. This could mean that many of the assumptions about epigenetic modifications in the brain based on peripheral tissue studies are not entirely accurate and need to be explored further.

Table 1: Comparison of Epigenetic Mechanisms Across Disorders

Epigenetic Mechanism	**Depression**	**Bipolar Disorder**	**Anxiety**
BDNF methylation	Hypermethylation; reduced BDNF levels during depressive episodes	Hypermethylation (stronger in BD II)	Hypermethylation (particularly in women)
SLC6A4 methylation	Hypermethylation in peripheral tissue; inconsistent in pregnant women & adolescents	Hypermethylation in bipolar twin in monozygotic twins discordant for BD	Methylation changes after CBT; contradictory directionality
FKBP5 methylation	Observed; stress-related	Hypermethylation observed	Hypermethylation observed; changes after CBT

H3K4 trimethylation	Increased at synapsin genes	Increased at SYN1-3	Increased in fear and stress-related brain regions (rodent)
HDAC expression / acetylation	Decreased HDAC2	Increased HDAC4; decreased HDAC6 & HDAC8	HDAC1 & 2 increased (rodent); HDAC3 unchanged
ncRNA alterations	miRNA downregulation in cortex & blood	lncRNA downregulation (male-specific)	miRNA dysregulation; limited specific overlap

Overall, several similarities were observed between the studied disorders including *BDNF* methylation, SLC6A4 variations, and miRNA levels. These helped find similarities between the disorders and also understand the effects of these modifications on the body based on existing understanding of symptoms. Despite the promise of certain epigenetic modifications being used as biomarkers, very few of these findings have been corroborated. The next step would be to deepen the understanding into the differences in these modifications across disorders and better define the effect on various biological pathways in order to use them as reliable differentiating markers to recognise these disorders early on.

Table 2: Convergent Pathways Across Depression, Bipolar Disorder, and Anxiety

Biological Pathway	**Marks Involved**	**Analysis**
Neuroplasticity	BDNF, SYN1-3, MeCP2	Linked to depressive episodes & mood regulation, appear to impair neural adaptability
Serotonergic signalling	SLC6A4, 5-HT3AR, raphe miRNAs	Highly state-dependent, temporal, and dependent on demographic and environmental factors
HPA axis stress response	NR3C1, FKBP5	Strong stress-environment interaction through altered hormonal regulation
Chromatin regulation	HDAC1-3, HDAC4, HDAC6, HDAC 8, H3K4me3	Universally implicated but highly isoform-specific and non-uniform

Non-coding RNA regulation	miRNAs, lncRNAs	Potential association with increased neurotransmission but variance in findings suggests currently underdeveloped evidence

The sample sizes of many of the studies are not large enough, providing data sets that are sometimes too small to determine consistent patterns. Additionally, there is a population bias, due to the fact that many epigenetic modifications need to be observed in the brain, which is difficult. Because of this many of the studies have been conducted on post-mortem brain tissue, which could differ from living tissue and its epigenetic characteristics. The use of rodent subjects, though useful in making observations that may not be possible in human studies, also limits the applicability of the findings as rodent populations show far less variability than humans, which makes the study of disorders far more complex and less consistent in humans.

While some of the studies were longitudinal, a majority have been short term, cross-sectional studies. Considering the role of factors such as environment and lifestyle in epigenetics, longer term studies could give greater insight into the direct cause and effect relationships between these factors and various epigenetic modifications, aiding in preventative measures. In that same vein, there is scope for conducting studies into specific environmental influences like diet, exercise, medication, pollution, and trauma that impact epigenetic mechanisms to better understand the environment-gene connection in the onset of mood disorders.

The study of epigenetics shows great promise in helping us answer some of the most relevant questions in Biology, Neuroscience, and Psychiatry. The progress and technological advancements in the field thus far have already presented valuable insights into highly prevalent conditions affecting the population today. But there is a long way to go, and further research can provide better understanding and aid in the management of various disorders, ensuring wide-spread benefit in a world where the frequency of these disorders is only increasing. Overall, epigenetics is a highly relevant, contemporary field of study that is sure to see growth and greater application in the coming years.

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