Potential Treatments for Epigenetic Dysregulation in Neurological Diseases

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

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Ethics Statement

This study does not involve any kind of human or animal trial.

Abstract

Epigenetics is of great importance to modern neuroscience because of its link to the etiology of certain neurodegenerative diseases. The roles of epigenetic changes like DNA methylation and histone acetylation in transcriptional control and dysregulation have been extensively investigated. DNA hypo methylation in Alzheimer's disease leads to elevated presenilin 1 expression, which in turn increases amyloid beta peptide deposition. Increased -synuclein expression and accumulated SNCA protein are hallmarks of Parkinson's disease, which is caused by aberrant methylation. Mutant Huntingtin causes transcriptional dysregulations in Huntington's disease by inhibiting histone acetyltransferases. This project paper reviews on the topic of epigenetics and neurodegeneration and discuss the role that how epigenetic dysregulation plays in the onset of these diseases and offer some insight into the therapeutic potential of epigenetics-based approaches.

Keywords

Epigenetics; Dysregulation; Methylation; Acetylation; Alzheimer’s disease; Parkinson’s disease; Huntington’s disease; Epidrugs

Dedication

I dedicate this dissertation to

my beloved parents,

without whom I would not have progressed this far

Acknowledgement

I would like to start by saying that I will always be grateful and honored to have had the chance to study at a prestigious university like BRAC University and to have had the opportunity to conduct research while being supervised by one of the kindest and most supportive faculty members, Dr. Sabrina Sharmin, Assistant Professor at the School of Pharmacy, BRAC University, who gave me unwavering support and encouragement. Her exceptional tolerance, consideration, and assistance throughout this time made my thesis project conceivable.

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List of Acronyms

AD: Alzheimer’s Disease

APP: Amyloid Precursor Protein

BBB: Blood-Brain Barrier

CNS: Central Nervous System

ChIP: Chromatin immunoprecipitation

DNMTi: DNA Methyl Transferase inhibitors

EGCG: Epigallocatechin 3-gallate

FDA: Food and Drug Administration

FISH: Fluorescent in situ hybridization

HD: Huntington's Disease

HDMi: Histone Demethylase inhibitors

HMTi: Histone Methyl Transferase inhibitors

HATi: Histone Acetyltransferase inhibitors

HDACi: Histone Deacetylase inhibitors

HATs: Histone Acetyl Transferases

HMTs: Histone Methyl transferases

HTT: Huntingtin

miRNAs: microRNAs

NGS: Next-generation sequencing

ncRNA: non-codingRNA

lncRNA: long non coding RNA

NFTs: Neurofibrillary Tangles

PAHs: Histone acetyl-binding proteins

PKC: Protein kinase C

PD: Parkinson’s Disease

PMHs: Histone Methyl-binding proteins

REST: Repressor Element 1 silencing transcription factor

ss-siRNAs: single-stranded siRNAs

SAM: S-adenosyl methionine

TETs: Tetramethylenedisulfotetramine

LSD1: Lysine-specific demethylase1

Chapter 1: Introduction

1.1 Background

Conrad Waddington used the word "epigenetics" to describe a theoretical approach to a crucial component in developmental biology. Almost all of a person's cells share the same DNA sequence. To provide just one example, whereas the DNA of liver cells and muscle cells is identical, the gene products they create couldn't be more different from one another (Coppedè, 2014). That similar genes may be variably translated into distinct cell types and specify distinct cellular activities requires a degree of biological activity that operates above ('epi') the genes (Hwang et al., 2017). Epigenetics, as described by Waddington in the 1940s (Landgrave-Gómez et al., 2015), is the study of the many interactions between genes and their products that ultimately lead to the manifestation of phenotype.

Embryonic development and cell differentiation rely heavily on epigenetics, which entails the heritable alteration of gene expression independent of the DNA sequence (Coppedè, 2021). In order to alter gene expression, epigenetic modifications such as DNA methylation, hydroxymethylation, nucleosome positioning, histone acetylation, deacetylation, phosphorylation, ubiquitination, and non-coding RNAs (such as microRNAs and long non-coding RNAs) build a complex network of covalent modifications to DNA cytosine bases and histone proteins (Figure 1). Epigenetic alteration modifies the availability and efficacy of transcriptional factors by altering chromatin compaction and histone protein binds to DNA promoter regions of neighboring nucleosomal core (Z. Xu et al., 2012).

Although the importance of epigenetics in human illness was initially recognized in cancer, it has now been extended to many other fields, including neurology, immunology, and metabolic diseases (Z. Xu et al., 2012). Recent studies have highlighted the importance of epigenetic controls in the nervous system, namely in the control of neuronal gene expression, genome

stability, and DNA damage (Rudenko & Tsai, 2014). Neurological diseases including Alzheimer's, Huntington's, Parkinson's, epilepsy, and multiple sclerosis are quickly gaining attention as potential sites of epigenetic changes (Gangisetty & Murugan, 2016). Currently present treatments for neurological illnesses just slow down the deterioration of symptoms for a short period of time (Coppedè, 2014).

Since epigenetic-based medications have shown surprising therapeutic results in neurological illnesses, scientists have begun to investigate the epigenetic process in neurodegenerative diseases (Z. Qi et al., 2021). Epigenetic-based medications have been proven to be potentially important in easing cognitive symptoms and avoiding or restricting motor symptoms in animal models of neurodegenerative disorders, providing the foundation for a potential deployment in human pathology (Coppedè, 2021). There are several histone deacetylation and DNA methylation inhibitors that have been licensed by the US Food and Drug Administration (US FDA) and used in clinical settings for quite some time. Recent research has focused on histone methylation and the function of microRNA in controlling gene expression as possible therapeutic targets (Kelly et al., 2010).

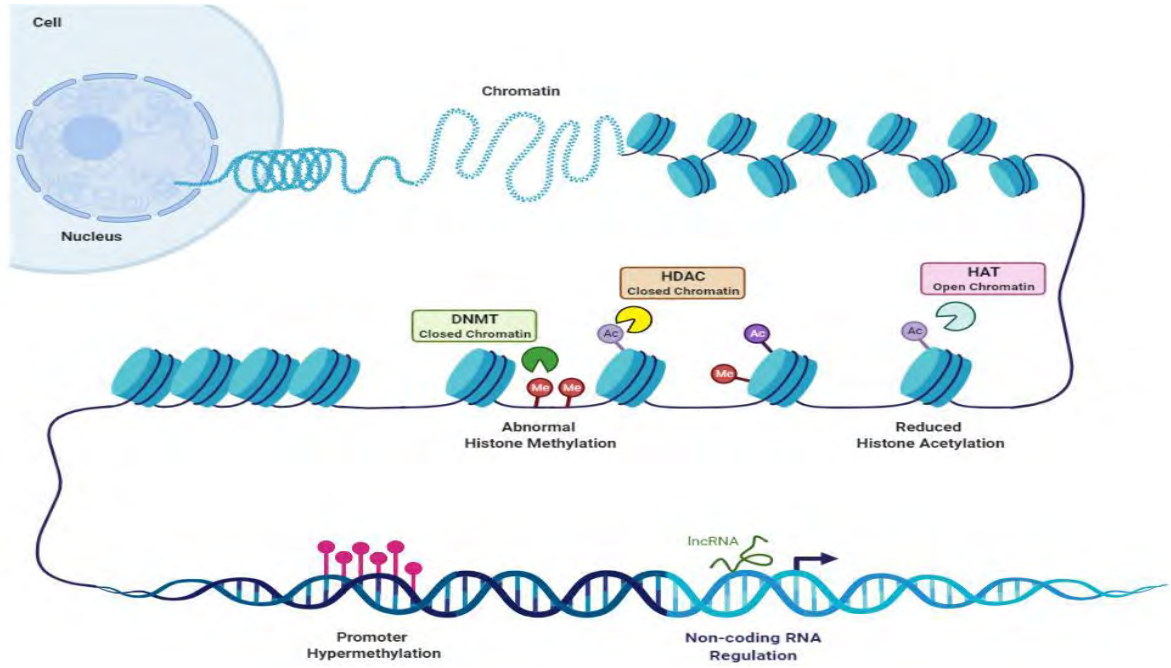

Figure 1: Schematic representation of the epigenetic modification in genome (Adapted from Conboy et al., 2021). DNA methylation, histone methylation, acetylation, and non-coding RNAs are some major epigenetic mechanisms that regulate gene expression and suppression.

1.2 Rationale of the Study

Epigenetic alterations have emerged as a key player in the development of sophisticated neuronal functions in the last several decades. Some forms of dementia may have their origins in the complex interplay between genes and the environment, and epigenetics may provide us with the missing piece of the jigsaw. To better understand the biology of these illnesses and, ultimately, to identify new therapy options, an understanding of the role of epigenetic alterations in neurodegeneration is essential. With a better understanding of the role epigenetic modifications play in the onset of these prevalent neurodegenerative illnesses, we can more effectively prevent, diagnose, and treat them. New epigenetic therapeutic targets are being considered, which might open the way for treating these disorders in the near future, thanks to a better knowledge of the epigenetic alterations that cause them.

1.3 Aim of the Study

Neuroscientists have come a long way in understanding the importance of epigenetics in both normal brain function and the development of neurodegenerative disorders. The goals of this literature review are to:

- Summarize alternative modes associated with epigenetic regulation of genes.
- Discuss the importance of maintaining epigenetic signatures throughout the lifetime.
- Provide an overview of the effect of epigenetic dysregulation in the pathology of neurodegenerative disorders.
- Emphasis on the promise of epigenetics-based therapies for neurodegenerative disorders.

Chapter 2: Methodology

An outline was made to convey the material in a methodical way, and this was done before beginning the literature research and topic selection. Secondary research articles were searched for relevant information using the terms "Epigenetic Dysregulation," "Neuroepigenetics," "Neurodegenerative Disorders," "Alzheimer's Disease," "Parkinson's Disease," "Huntingtin Diseases," "Epigenetic Therapy," and "Epigenetic Drug" in databases like PubMed, Scopus, and Google Scholar. Compilation, summarization, and referencing of the articles proceeded after the articles were evaluated to extract the necessary data according to the planned outline. This study makes use of a number of different pieces of software, such as ChemDraw for depicting the medications' chemical structure, BioRender for depicting the figures and diagrams, and Mendeley for creating in-text citations and a bibliography.

Chapter 3: Epigenetics

Epigenetic processes control genome shape and function based on external cues. Epigenetic regulation relies on DNA methylation, histone modification, chromatin remodeling, and non-coding RNA (Qureshi & Mehler, 2013). Epigenetic processes alter chromatin structure or disclose regulatory subunit binding sites to influence gene expression (Kofink et al., 2013). Gene expression, transcription, RNA processing, translation, DNA repair, genomic imprinting, genomic integrity, and X chromosome inactivation are carried out by such dynamic and highly linked mechanisms (Qureshi & Mehler, 2014).

3.1 Epigenetic Modifications

3.1.1 DNA Methylation/Hydroxymethylation Methylation

DNA methylation chemically transfers a methyl group to the carbon fifth position on the cytosine residue of a pyrimidine ring in the CpG background. DNA methyl transferases (DNMTs) use S-adenosyl methionine (SAM) to covalently add methyl groups to cytosine bases (Figure 2). (Gangisetty & Murugan, 2016). CpG islands, dense CG dinucleotide repetitions that affect gene expression by binding transcription factors to the promoter region, are most commonly methylated. DNA methylation was aided by methyl transferases. DNMT1, DNMT3A, DNMT3B, DNMT3L, and DNMT2 are five DNA methyl transferases enzymes that establish and maintain DNA methylation. DNMT3a and DNMT3b establish the DNA methylation pattern during early development, and DNMT1 maintains it during DNA replication in subsequent cell generations. DNMT2 methylates RNA, unlike DNMT3a, 3b, and 1. (Bertogliat et al., 2020; Z. Xu et al., 2012).

DNA Hydroxymethylation

TETs oxidize 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC) in DNA hydroxymethylation (Figure 2). 5-hydroxymethylcytosine (5-hmC) is abundant in euchromatin

and implicated in transcription. In the methylated CpGs area of the genome, translocase dioxygenases (TETs) enzymes block methyl transferase association with DNA and allow demethylation by oxidizing 5-hmC to produce unmethylated DNA through base excision (Bertogliat et al., 2020).

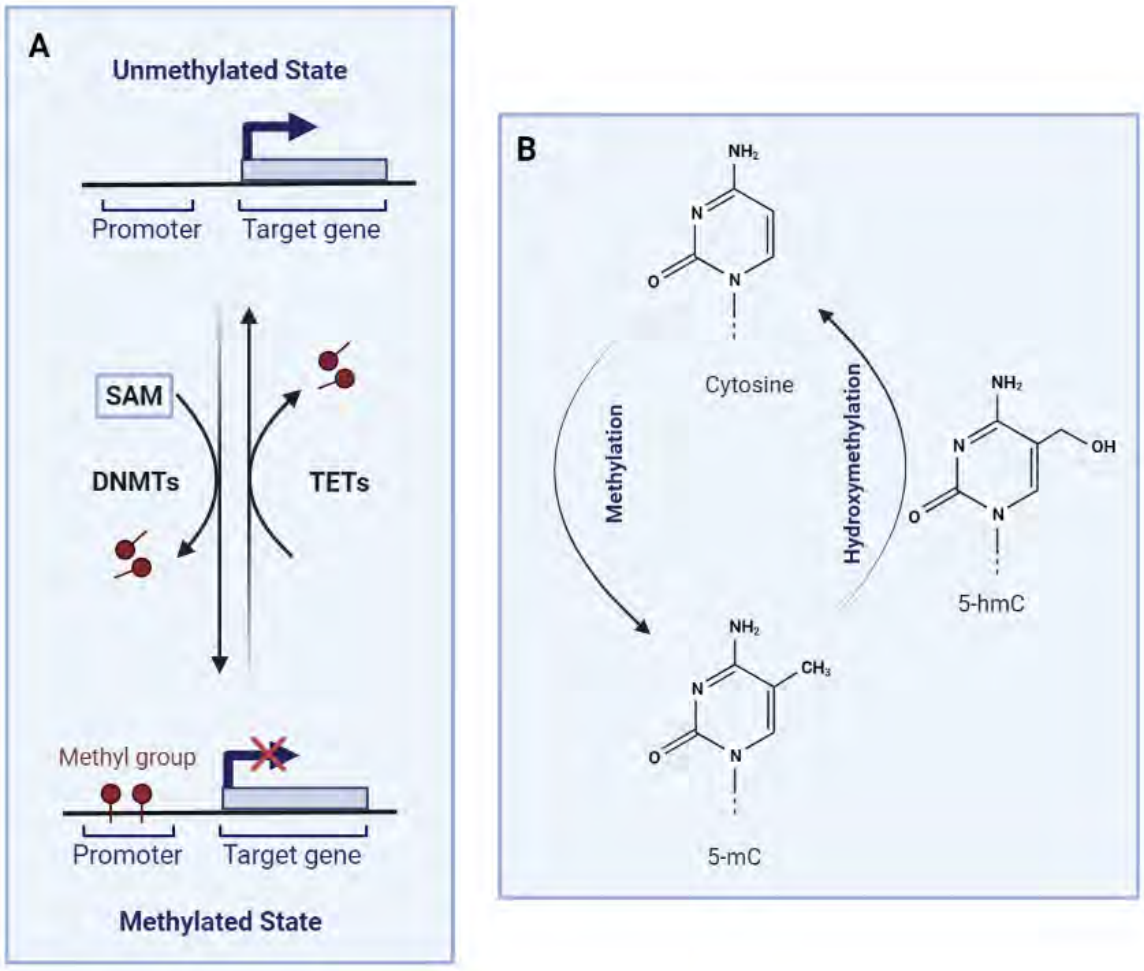

Figure 2: Methylation and hydroxymethylation of the DNA (Adapted from M. Xu et al., 2021). A) DNA methylation of the promoter region of a gene catalyzed by DNMTs inhibit the transcription activity and in hydroxymethylation, TETs catalyze the removal of the methyl group from the promoter region and restore the gene transcription. B) DNA methylation involve covalent addition of a methyl group at the fifth carbon on the cytosine base and alternatively, in DNA hydroxymethylation, the methyl group from the fifth carbon is removed to establish a demethylated cytosine base.

3.1.2 Histone Modification

The building blocks of chromatin are nucleosomes, which consist of DNA wrapped around histone proteins. Histone proteins, known as octamers, are found in close proximity to DNA.

Each octamer contains two copies of H2A, H2B, H3, and H4. Post-translational modifications to histones are a signature feature of chromatin remodeling in the brain. Some amino acid residues in the N-terminal tail of histones are accessible to acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. These include lysine, arginine, serine, and threonine. The formation of these modifications can either activate or repress transcription, depending on the residue's position and the type of alteration. So, this is what the histone code consists of (Bertogliat et al., 2020; Gangisetty & Murugan, 2016).

Modifications to histones involving acetylation and deacetylation

Positive transcription of the genome is regulated by acetylation of histones. Lysine acetylation on histones is a well-documented epigenetic change. Acylation of histones H3 and H4 leads to an open chromatin structure, which stimulates gene expression. Dynamic regulation of acetylation of histone residues is mediated by histone acetyl transferases (HATs) and histone deacetylases (HDACs). Catalyzing the transfer of acetyl groups from acetyl coenzyme A to histone lysine residues, histone acetyl transferases (HATs) are the primary enzymes involved in the process of histone acetylation (Figure 3). The positive charge of histones is neutralized by the transfer of acetyl groups, which therefore allows for an open chromatin conformation and the subsequent activation of transcription. Alternately, histone deacetylation occurs when histone deacetylases (HDACs) enzymes catalyze the removal of acetyl groups from lysine residues in the conserved tails of histone proteins, resulting in a net positive charge. The creation of the most compact chromatin structure, engaged in transcriptional repression, is facilitated by the strong contact between the negatively charged DNA and the positively charged histones (Gangisetty & Murugan, 2016).

Reversible Methylation and Demethylation of Histones

Most commonly seen on arginine and lysine residues, histone methylation has been connected to a variety of processes, such as transcriptional activation and repression (Landgrave-Gómez et al., 2015). Mono-methylation, di-methylation, and tri-methylation typically take place at arginine and lysine residues. Histone methyl transferases (HMTs) enzymes utilizing SAM as a methyl donor catalyze the methylation modification of H3 and H4 amino acid side chains (Figure 3). Expression of a gene is either silenced or activated depending on the level, symmetry, and location of histone methylation. Although histone methylation does not change the overall shape of chromatin, it does facilitate the regulation of gene expression by other proteins. The process of histone demethylation is also crucial in controlling gene expression. The enzyme lysine-specific demethylase1 (LSD1) is the first of the histone demethylase family to be able to remove both mono- and di-methyl groups from DNA (Gangisetty & Murugan, 2016).

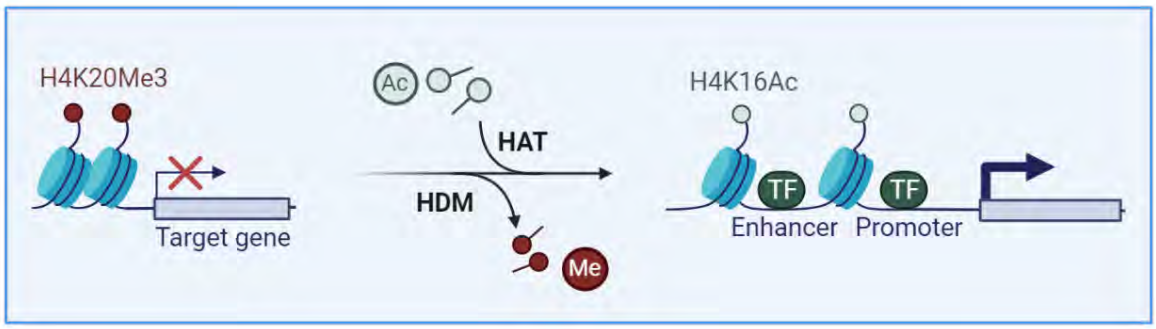

Figure 3: Histone acetylation and methylation on lysine residues (Adapted from Bannister & Kouzarides, 2011). Histone acetylation performed by HATs leads to opening of chromatin that allow transcription factors to bind on the promoter region and induce target gene expression. Conversely, histone demethylation, performed by HDMs leads to condensation of chromatin, thereby prevent the binding of transcription factor and repressing the target gene expression.

3.1.3 The Role of Non-Coding RNA in Disease

Important for understanding gene expression at both the transcriptional and post-transcriptional levels, non-coding RNAs (ncRNA) are functional RNAs that do not convert into proteins (Figure 4). Micro, short-interfering, circular, long non-coding, and PIWI-interacting RNA are

just a few examples of the ncRNA with epigenetic connections. Most research on ncRNA has focused on microRNAs (miRNAs) and long noncoding RNAs (ncRNA) because of their potential significance in neurological disorders (Bertogliat et al., 2020).

By binding to the 3' untranslated region (3' UTR) and degrading or silencing transcripts, microRNAs (miRNAs) control the post-transcriptional production of mRNAs. Numerous therapeutic options for human disease are made available by the ability of a single miRNA to work on multiple genes, creating a highly complex epigenetic environment (Bertogliat et al., 2020).

Transcripts of RNA longer than 200 nucleotides, known as long non-coding RNA (lncRNA), are abundant in brain tissue. Evidence suggests that lncRNA regulate gene expression by participating in chromatin remodeling, which they do by targeting certain histones or DNA locations with a variety of modifying proteins. It's also possible for lncRNA to play a role in the production of antisense transcripts, which can hide genes from view and shield their sequences from degradation by microRNAs (Bertogliat et al., 2020).

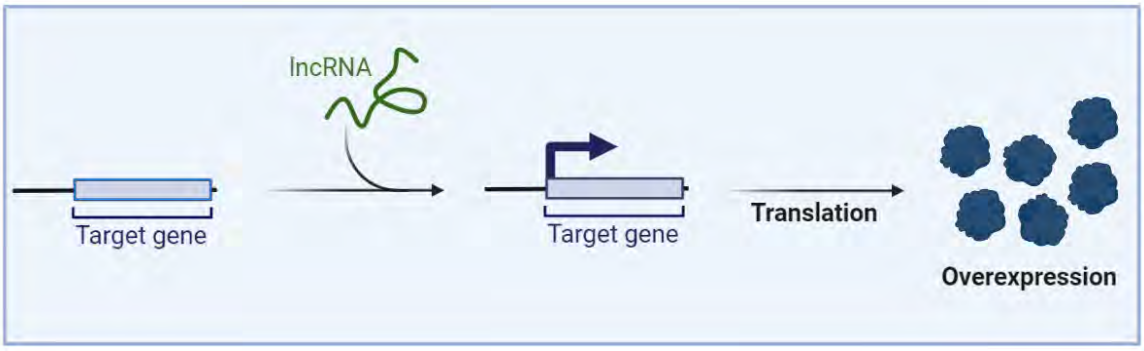

Figure 4: Non-coding RNA (ncRNA) mediated gene regulation (Adapted from Kumar et al., 2020). Non-coding RNAs including lncRNA can interact with genes or gene product and influence expression of certain proteins.

3.2 Regulation of Epigenetics During the Human Life Cycle

The field of epigenetics studies the intersection between genetics and environmental influences. Endogenous factors, such as synaptic changes, hormone shifts, and drug responses, as well as exogenous factors, such as exercise, food, stress, and environmental disturbances,

all have a role in regulating gene expression (Figure 5). (Cacabelos & Teijido, 2018). Dynamic and plastic reactions to intra- and extracellular stimulations, mediated by cell-cell interaction, surrounding cells, individual physiology, or the exposed environment, characterize epigenetic alterations. Several environmental influences, such as growth factors, cytokines, hormone levels, and stress-response and neurotropic factors, might modify epigenetic expression. Enzymes add or remove epigenetic marks on DNA and histone proteins in response to these epigenetic alterations, setting off a cascade of changes in cellular activity that might be transient, permanent, or heritable (Kanherkar et al., 2014).

In many respects, epigenetic expression can be equated with genomic "software," as it controls both prenatal and postnatal development. Epigenetic modifications have been found to remain "plastic" throughout all stages of brain development and aging, with evidence indicating that these dynamic alterations occur even in neurons and other post mitotic brain parts (Jakovcevski & Akbarian, 2012). The epigenome’s malleability enables for "learning" from experience and adaptation to new conditions. At various points in development, epigenetic alteration activates or represses distinct sets of genes; these genes, in concert with external cues, determine how much and how quickly a person grows and develops. Any epigenetic factor that interferes with gene transcription at any point in a person's lifecycle has the potential to disrupt the regulating system, with potentially lasting effects.

While the ability to adjust to new circumstances is helpful, it also makes it easier for inherited and acquired diseases to take hold. In order for cells derived from the embryo to emerge with cell-specific gene expression patterns and retain adequate differentiation, the majority of genes must undergo complete reprogramming immediately after conception to produce an epigenetic "clean slate." If something goes wrong during this time, the child's phenotype could be altered permanently. While internal body signals continue to play a role in developmentally important

processes like learning and physical growth, other, more distal, external influences from the environment and society also begin to have an effect (Kanherkar et al., 2014).

Epigenetic responses to environmental factors, such as food, have been shown through genomic imprinting studies to influence illness onset. Folic acid, B vitamins, and s-adenosyl methionine (SAM) are all crucial nutrients that contribute to the creation of the methyl group, which is required for the formation of important epigenetic modifications for gene silencing. Alternate expression of genes encoding growth factors can result from deficits in folate and methionine, two amino acids that provide the methyl groups necessary for methylation. The global DNA methylation level is critical in the pathogenesis of autism spectrum disorder, and a study on mouse offspring showing the effects of maternal folic acid supplementation found a significant difference between the groups fed folic acid and those given a relatively low dose of folic acid supplements (Kanherkar et al., 2014).

Diseases can be passed on to future generations by fetal exposure to toxic chemicals, pesticides, and other environmental hazards due to methylation of genes. Toxic heavy metals found in the environment can change DNA methylation and histone acetylation. As a result, these have been linked to a wide range of illnesses, including cancer, autoimmune diseases, and neurological disorders. Possible processes include metal catalyzing oxidation events, which degrade biological macromolecules, generate free radicals, and cause epigenetic modification. The epigenome is altered by recreational drugs like alcohol, nicotine, cocaine, opiates, and amphetamines through changing the methylation level in brain regions like the nucleus accumbens, the primary pleasure reward center. Changes in DNA methylation and other epigenetic changes that regulate gene expression are influenced by smoking. Cigarette smoking, for instance, demethylates DNMT3B expression, which in turn demethylates the 13 metastatic genes of lung cancer cells. There is evidence that alcohol consumption lowers tissue

SAM levels, which in turn leads to DNA hypo methylation and site-specific histone acetylation, methylation, and phosphorylation (Kanherkar et al., 2014).

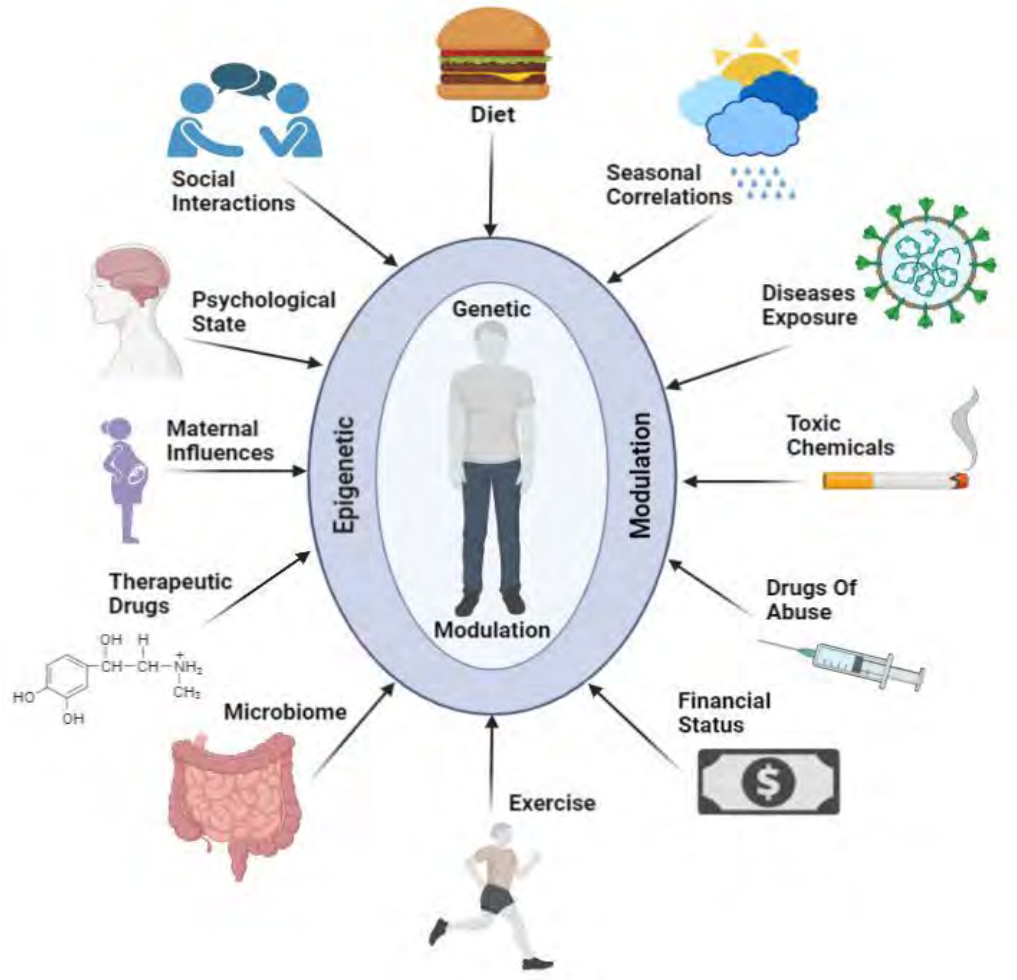

Figure 5: Epigenetic modification through different factors (Adapted from Kanherkar et al., 2014). Different factors including diet, disease exposure, toxic chemicals, therapeutic drugs and maternal health can induce epigenetic modifications and thereby affect gene transcription.

3.3 Diseases and Epigenetic Alterations

Because epigenetics provides an additional explanation for illness pathogenesis that cannot be characterized solely by genetic or environmental factors, it has contributed to the advancement of medical science (Oh & Petronis, 2021). Epigenetic regulation of gene expression profile is initiated and maintained by major epigenetic mechanisms such as DNA methylation, demethylation, histone modification, and non-codingRNA (ncRNA). Epigenetic alterations play an important role in ensuring normal human biological development, as evidenced by the

appearance of various disorders when the wrong types of epigenetic marks are incorporated at the wrong time or in the wrong place (Portela & Esteller, 2010). Mutations in the various genes that code for epigenetic regulators can lead to a wide variety of illnesses (Murgatroyd, 2016). Epigenetic regulation of gene expression is implicated in the development of autoimmune diseases, cancer, and other pathologies (Zhang et al., 2020).

Epigenetic alteration can happen via a direct or indirect mechanism. It appears that direct modification occurs whenever a factor alters the structure of epigenetic enzymes in some way other than by attaching to and blocking their normal activity, leading to malfunction, or upregulating their expression. Next, there is a shift in the bioavailability of epigenetic enzymes, which results in the incorrect addition of epigenetic tags to enhancers and promoters across the genome. These modifications may have an indirect influence on the entire genome, altering the epigenome in a way that does not target any specific gene. The availability of substrates, intermediates, by-products, or any other component involved in the metabolic route necessary to create epigenetic tags can be directly modified whenever a factor influences the pathway. Next, it alters the supply of epigenetic tags, leading to widespread epigenome reprogramming. Indirect mechanisms are the other major way in which variables may influence epigenetic expression. According to the second process, transcription factor activity is affected after short-term exposure to a factor due to changes in the expression of receptors and growth factors. The transcriptional and genetic regulatory proteins undergo alterations in gene expression in response to chronic exposure to the stimuli, which in turn affects the activity of the enzymes that add or remove epigenetic tags from chromatin (Kanherkar et al., 2014).

3.4 Epigenetic Pattern Molecular Profiling

There are a variety of time-tested methods for estimating or precisely calculating DNA methylation that can be used. The most often used method is bisulfite conversion, which is also

widely regarded as the gold standard because of its relatively high resolution, 1 bp. For epigenetic regulation, 5mC is the most prevalent form of DNA methylation; the bisulfite approach distinguishes 5mCs from unmethylated cytosines by treating genomic DNA with sodium bisulfite, which deaminates unmethylated cytosines to uracil while leaving methylated cytosines unaltered. DNA methylation can also be ascertained by enriching methylated DNA and then examining the enriched sequences with another method, such as microarrays or sequencing. Immunoprecipitation with an antibody (i.e., methylated DNA immunoprecipitation) that can detect methylated cytosines selectively can be used to enrich for methylated DNA (Li, 2021; Qureshi & Mehler, 2011). Assessing nucleosome dynamics and chromatin accessibility, as well as histone modifications and proteins involved in chromatin structure, can all be done using a variety of methods. Chromatin immunoprecipitation (ChIP), an efficient method for identifying DNA-protein interactions, is important to these approaches by enriching genomic sequences that are linked with particular chromatin configurations. In chromatin immunoprecipitation (ChIP), an antibody identifies an epitope consisting of a specific histone alteration. The histone modification distribution across the genome can be mapped by analyzing the DNA sequences present in ChIP-enriched DNA. It is possible to visualize the relationship between histone modification patterns and the spatial layout of chromosomes in three dimensions (3D) using ChIP-sequencing in conjunction with 3C-based techniques such as Hi-C. (Li, 2021; Qureshi & Mehler, 2011). Numerous biological samples, especially those from the circulatory and nervous systems, have been examined for their non-coding RNAs (ncRNAs). Large-scale profiling of ncRNAs is now carried out using either microarrays or next-generation sequencing (NGS). In addition, tissue samples can be analyzed for their RNA origin using fluorescent in situ hybridization (FISH). Techniques including quantitative real-time polymerase chain reaction (qRT-PCR) and northern blotting were employed to confirm initial findings during the discovery phase (Snijders et al., 2018).

Chapter 4: Epigenetic Dysregulation in Neurodegenerative Diseases

4.1 Epigenetic Dysregulation in Alzheimer's Disease

4.1.1 Pathogenesis of Alzheimer's Disease

According to the amyloid-cascade hypothesis, amyloid beta (A) is the first step in the pathogenic pathway that leads to neurodegeneration and amyloid peptide accumulation in Alzheimer's disease (AD). Different cells produce the transmembrane protein amyloid precursor protein (APP). Two distinct routes exist in the central nervous system for the sequential cleavage of APP. In the first, non-amyloidogenic pathway, APP is cleaved by -secretase to generate sAPP, an extracellular secreted protein, and C83, a membrane-bound 83-amino acid fragment. The intracellular domain of APP remains attached to the membrane after -secretase cleaves it at position 83, but a new secreted fragment known as p3 is produced (AICD). -secretase, also known as BACE-1 (-site APP cleavage enzyme 1), is the first enzyme involved in the second, amyloidogenic pathway, where it cleaves APP to generate sAPP, an extracellular secreted product, and C99, a membrane-bound fragment of APP's C terminus consisting of 99 amino acids. A and the identical membrane-bound APP intracellular domain are secreted from the cell after C99 has been digested by -secretase (AICD). If the typically soluble A is not cleared away quickly enough, it will self-assemble into oligomers, and then into the extremely regular amyloid fibrils that make up the plaques. When C99 is cleaved by -secretase, the resulting A peptide is between 37 and 42 amino acids in length. It has been found that the longer variants (A40 and, especially, A42) are more prone to self-aggregation and fibril formation than the shorter forms. As with amyloid plaques, soluble A is toxic and is thought to affect cellular functions through secondary events such as hyperphosphorylation of the tau protein, which results in the formation of neurofibrillary tangles (NFTs), excitotoxicity, oxidative stress, and inflammation (Soria Lopez et al., 2019).

There is evidence that mutations in the presenilin (PS) genes PS1 and PS2 contribute to the development of Alzheimer's disease. The -secretase proteins linked to PS1 and PS2 genes are involved in the generation of the A peptide from the precursor protein amyloid precursor protein (APP). Increased production of A42, the longer isoform of A that is more prone to aggregate than the shorter isoform A40, results from mutations in the PS genes, while decreased production of A40 results in the opposite effect. Particularly the e4 allele of the apolipoprotein E (APOE) gene, which confers the highest risk in the population, and certain mutations in the PS and APP genes appear to have a substantial role in illness etiology. The CLU gene has also been linked to Alzheimer's disease because it is thought to be responsible for the aggregation and clearance of A. In addition to this, malfunctioning tau has been linked to the development of AD. Tau is hyperphosphorylated in Alzheimer's disease. Tau is a microtubule-related protein that helps with microtubule assembly. Therefore, it becomes detached from microtubules and aggregates, leading to cytoskeletal disorganization, neuronal malfunction, and cell death, all of which have been linked to Alzheimer's disease pathogenesis (Lardenoije et al., 2015).

4.1.2 Role of DNA Methylation in Alzheimer's Disease

Amyloid beta protein (A) is a key constituent of senile plaque. A is generated when the enzymes -secretase (BACE) and -secretase (Secretion) sequentially degrade the amyloid precursor protein (APP) (presenilin). Beta-secretase cleavage produces A40 and A42, with the latter being linked to neurotoxicity. In familial Alzheimer's disease, mutations in the amyloid precursor protein (APP), presenilin 1 (PS1), and beta-secretase (BACE) (fAD). It has been proven that hypomethylation of the APP gene promoter leads to elevated levels of the APP protein, which in turn appears to boost the production of A. In vitro, hypomethylation caused by a lack of folate led to elevated levels of BACE and PS1 gene expression, but these levels normalized once SAM supplementation was added to the mix. These findings may suggest that

decreased DNA methylation of the APP, PS1, and BACE genes stimulates their transcription. Senile plaque forms when A production and deposition are boosted by a combination of these risk factors. Also, A has been shown to induce widespread hypomethylation of the DNA in cultured cells in the lab. Additional synergies may result from the A-induced genome-wide hypomethylation affecting the activity of other key AD-causing genes. For instance, hypomethylation promotes TNF- and caspase-3 expression, leading to an increase in TNF- and caspases production, which in turn promotes A expression and may initiate a cascade of unfavorable feedback loops (J. Wang et al., 2013).

A further pathological hallmark of Alzheimer's disease are neurofibrillary tangles, which are collections of intraneuronal paired helical filaments (PHF). Commonly, cellular microtubules serve as the primary structural element of NFTs; however, in AD, this component is replaced by tau protein, which has an aberrant phosphorylated structure. One of the key functions of tau, a phosphoprotein belonging to the microtubule-associated family, is to keep microtubules from becoming unstable. Degeneration of the cytoskeleton and neuronal death occur when tau becomes hyperphosphorylated, causing it to aggregate and lose its ability to attach microtubules. As tau became hyperphosphorylated, NFTs were produced. The methylation reaction in the cytoplasm is necessary for this phosphorylation event to occur. By blocking the methyltransferases and hypomethylating the protein phosphatase 2A (PP2A), which dephosphorylates the phosphorylated tau, an elevated homocysteine (Hcy) level has been shown to produce hyperphosphorylation of tau, the development of NFTs, and the production of SPs (J. Wang et al., 2013).

Studies have shown that the clusterin gene (CLU) is the third most prevalent risk gene associated with LOAD. There is strong evidence that the CLU gene, which is known to have a CpG-rich methylation area at its promoter 20 site, plays a crucial role in the pathogenesis of AD through its effects on A aggregation, clearance, and ultimately deposition. Patients with

Alzheimer's disease have been found to have greater concentrations of CLU in their brain and CSF. Recent research has shown that the plasma concentration of CLU is correlated with the severity of AD at the outset of the disease, the rate of clinical progression, and brain shrinkage (J. Wang et al., 2013).

4.1.3 The Role of Histone Alterations in Alzheimer's Disease

A key role for histone changes in neuronal development and Alzheimer's disease pathogenesis. Different disorders in signaling, proliferation, neural plasticity, apoptosis, inflammation, and immunology have all been linked to dysregulation of histone acetylation. There are 4,162 distinct acetylated markers on H3K27 in the brains of AD patients who have passed away. APP, PSEN1, and PSEN2 are only a few of the genes associated with AD pathogenesis that showed acetylation. The temporal lobes of those with Alzheimer's disease were shown to have lower levels of histone acetylation. In instance, compared to aged healthy controls, Alzheimer's disease brains had lower levels of acetylated lysine 16 on histone H4 (H4K16ac), which was involved in generating DNA damage and accelerated aging. Animal studies showed decreased acetylation of histone H3 at lysine 27 in regions related to plasticity (H3K27). Reductions in cognitive and synaptic activity in AD patients have also been linked to higher levels of histone deacetylases (HDACs), especially Class I HDACs (HDAC2 and HDAC3), the enzymes that catalyze the removal of acetyl groups and limit transcriptional activity by compacting chromatin. The hippocampus and cortex of AD patients, as well as animal models of the disease, were found to have elevated HDAC6 levels. Inflammation, tubulin acetylation, and tau phosphorylation and degradation have all been linked to the HDAC6 enzyme. Tau aggregation and neuronal survival may be improved by decreasing HDAC6 enzyme levels, which increases tau clearance. However, microtubule integrity, vesicular transport, and mitochondrial transport are all compromised by overexpression of the HDAC6 enzyme, which reduces acetylation of - 21 tubulin. Since elevated levels of HDAC4 promote apoptosis and

inhibition of HDAC4 reduces neuronal cell death, it is possible that HDAC4 plays an important role in nerve function. Class III histone deacetylase enzymes (HDACs), also known as sirtuins, have been linked to pathophysiology and are crucial in learning and memory. In addition, it was shown that the parietal cortex of the brains of AD patients had reduced levels of SIRT1. These alterations have been connected to amyloid-beta (A) and tau (tau) accumulation, as well as tau acetylation at lysine 28, which causes tau to aggregate in a major way. Higher trimethylation of lysine on histone H3 (H3K9), which is implicated in gene silencing and condensing heterochromatin structure, was detected in the postmortem brains of patients with Alzheimer's disease. Further, DNA damage has been linked to increased phosphorylation of serine on histone H3 (H3S10) in hippocampal neurons and astrocytes (Nikolac Perkovic et al., 2021).

4.1.4 AD and noncoding RNAs

Elevated levels of APP expression are associated with increased levels of A production, which in turn contribute to the pathophysiology of Alzheimer's disease. Extensive in vitro investigations have identified many microRNAs (miRNAs) that regulate the expression of amyloid precursor protein (APP) mRNA. Evidence suggesting miR-101 plays a crucial role in the control of APP expression and A production in AD has shown that it is highly expressed in the anterior temporal cortex of the human brain. The levels of amyloid beta (A) and amyloid precursor protein (APP) in hippocampal neurons were significantly reduced after miR-101 overexpression was inhibited. Overexpression of miR-16 in vitro and in vivo reduced APP protein expression, suggesting that miR-16 may also target APP and have an effect on the development of AD in mice. Patients with AD had lower levels of miR-124 expression, highlighting the importance of the miR-124 pathway in controlling APP splicing in neurons. The expression of BACE1, which is strongly linked to cell death due to A neurotoxicity, was

found to be under the control of miR-124. Suppressing or overexpressing miR-124, on the other hand, could lead to a rise or fall in BACE1 expression, respectively (J. Wang et al., 2013).

Normal synaptic, cognitive, and emotional functioning rely on -secretase BACE1, the rate-limiting enzyme in A synthesis. BACE1 mRNA expression has been shown to be controlled by a number of microRNAs (miRNAs), including miR-9, miR-29a/b-1, miR-29c, miR-107, miR-298, -485-5p, and lcRNAs such BACE1-antisense (BACE1-AS). Overexpressing transient miR-29a/b-1 lowered BACE1 and A levels. The finding that miR-107 is downregulated at several points in the AD pathogenesis suggests that it may play a major role in hastening the progression of the disease via controlling BACE1. Last but not least, microRNAs miR-298, miR-328, and miR-195 have been demonstrated to decrease the amount of A in neural cultured cells by inhibiting BACE1 translation. Furthermore, in neural cultured cells, miR-125 expression was reduced while BACE1 expression was elevated (J. Wang et al., 2013).

Multiple studies have found that microRNAs play a crucial role in Tau's metabolic activities, which in turn lead to various neuropathological features of Alzheimer's disease. Hypophosphorylation of neuronal tau could have resulted from a decrease in miR-15 levels, which may have affected the expression of extracellular signal-regulated kinase 1 (ERK1), a direct tau kinase, in mouse brain cells. Another member of the tau kinase family, GSK-3', plays a crucial role in amyloid- (A) production and NFT maturation. In smooth muscle, BDNF controls miR-26a's suppression of the GSK-3' mRNA. Additionally, AD is associated with a shift in the expression of miR-26a. Because SIRT1 may deacetylate tau, its absence promotes Tau acetylation and results in the accumulation of hyper phosphorylated Tau in Alzheimer's disease. Each miRNA, miR-9, -34c, and -181c, has been specifically chosen for its ability to significantly repress SIRT1 mRNA and demonstrate associated dysregulation in AD brain (J. Wang et al., 2013).

4.2 Disrupted epigenetic control contributes to Parkinson's disease

4.2.1 The Determinants of Parkinson's Disease

Motor problems such as tremor, bradykinesia, stiffness, and gait difficulties are the most common and disabling for patients with Parkinson's disease (PD). Consequences in the realm of psychology, such as memory loss, inability to sleep, and impaired autonomic function, often follow physical difficulties in the form of dementia or other forms of motor dysfunction. Malfunctioning neurotransmitters in the serotonergic, cholinergic, and noradrenergic systems are linked to various mental health issues (Lardenoije et al., 2015).

Reduced numbers of dopaminergic neurons in the substantia nigra pars compacta are the primary pathology underlying Parkinson's disease (SNpc). Motor difficulties, bradykinesia and rigidity in particular, are linked to moderate to severe loss of dopaminergic neurons within the substantia nigra pars compacta area, as shown by clinical and pathological correlative research. The accumulation of misfolded proteins is a characteristic of neurodegenerative disorders, and Parkinson's disease is no exception. When it comes to Parkinson's disease, Lewy pathology is linked to the most important symptom. -synuclein, parkin, and ubiquitin are the main components of the protein aggregation known as Lewy bodies, which is located in the cytoplasm. When the protein is misfolded, it becomes insoluble and forms clumps with other misfolded proteins to form intracellular inclusions in the cell body (Lewy bodies) and processes (Lewy neurites) of neurons, which are notoriously difficult to clear. The sympathetic ganglia, vagus nerve, enteric nervous system, cardiac plexus, salivary glands, sciatic nerve, cutaneous nerves, and adrenal medulla are only some of the other parts of the nervous system that can be affected by Lewy pathology (Kalia & Lang, 2015).

Among the major risk genes for Parkinson's disease is synuclein alpha (SNCA), which encodes the presynaptic protein -synuclein. However, overexpression of the SNCA gene can only result

in familial Parkinsonian symptoms through point mutations and possibly duplications. Additional risk genes include leucine-rich repeat kinase 2 (LRRK2), microtubule-associated protein tau (MAPT), and Parkinson disease 16 (PARK16), with specific SNCA and MAPT SNPs carrying the greatest risk (Lardenoije et al., 2015).

4.2.2 DNA methylation in Parkinson's disease

DNA methylation is susceptible to influence from a wide variety of stimuli, especially those involved in the metabolism of a single carbon unit. Homocysteine must be converted to methionine and then to SAM, a universal methyl donor, and this process requires folates and vitamins. SAM donates a methyl group to form S-adenosylhomocysteine (SAH), which is further transformed to homocysteine. Dopaminergic neurons are vulnerable to injury when homocysteine levels are elevated and dietary folate intake is low, as seen in an animal model of Parkinson's disease. Some people with Parkinson's disease also have abnormally low levels of vitamin B12 and folic acid. Since aberrant DNA methylation may play a role in the etiology of Parkinson's disease, it follows that one-carbon metabolic dysregulation may play a role in the development of the disorder. Many neurodegenerative illnesses, including Parkinson's, have been linked to elevated levels of homocysteine. Increased homocysteine levels trigger oxidative stress, which in turn causes free radical production, mitochondrial malfunction, and, eventually, cell demise. Oxidative DNA damage, which is facilitated by free radicals, may increase with elevated homocysteine levels and lead to an increase in the frequency of mutations (Feng et al., 2015).

One of the most important risk genes for Parkinson's disease is alpha-synuclein (SNCA), whose activity is controlled by DNA methylation. Overexpression of SNCA may result from hypo methylation in its intron 1 in Parkinson's disease patients.

It was also shown that methylation at CpG-2 sites in the SNCA promoter was considerably lower in PD patients than in controls. Additionally, both the brains of SNCA animal models and postmortem brains with PD show a reduced nuclear DNMT1 level. This elucidates the molecular mechanism underlying decreased SNCA methylation. When SNCA accumulates and clumps in neurons, it disrupts the normal nuclear transit of DNMT1, resulting in DNMT1 delocalization to subcellular regions, where it is normally found. In addition, widespread hypomethylation of genes was discovered in the brains of PD patients with a lower amount of DNMT1 (Feng et al., 2015; Rathore et al., 2021).

One of the key risk factors in both Alzheimer's disease and Parkinson's disease is the microtubule-associated protein tau (MAPT), which encodes tau protein and aids in stabilizing the axonal cytoskeleton. Possibly by altering MAPT gene expression and hence influencing axonal cytoskeleton stability, hypermethylation of the MAPT gene is involved in the pathogenesis of Parkinson's disease. Another inflammatory response factor, tumor necrosis factor alpha (TNF-), has been linked to Parkinson's disease development (PD). There is a higher susceptibility of SNpc-area neurons to TNF-mediated inflammation in PD patients due to the hypomethylation of the TNF-promoter (Feng et al., 2015).

4.2.3 Histone Alterations in PD

3 Histone PD is characterized by aggregation of SNCA in the presynaptic nerve terminals of dopaminergic neurons in the substantia nigra (SN) of the brain. Changing the acetylation level of histones may mediate SNCA's neurotoxic effects. Furthermore, the SNCA locus was found to have an H3K27ac enrichment pattern (Feng et al., 2015; Rathore et al., 2021).

Dopaminergic neurons isolated from PD patients were found to have higher levels of acetylation on histone H2A, H3, and H4 compared to healthy controls. The accumulation of alpha-synuclein has been linked to an increase in histone H3 hypoacetylation, as indicated by

the research. The formation of alpha-synuclein is regulated by histone acetylation, while alpha-synuclein has been shown to regulate histone acetylation via a feedback mechanism. By shielding histone proteins and blocking histone acetylation, chromatin compaction, and gene expression, synuclein buildup in Parkinson's disease can lead to neurotoxicity and, eventually, cell death. In the development of protein aggregates that have folded incorrectly, histone deacetylase 6 plays a crucial role. This enzyme is involved in neuroprotection of dopaminergic neurons and is present in the Lewy bodies of PD pathogenesis. Mutations in genes related to histone deacetylase 6 may cause the accumulation of toxic synuclein oligomers and stimulate the creation of synuclein inclusions, which in turn protect dopaminergic neurons from synuclein toxicity (Feng et al., 2015; Rathore et al., 2021; Renani et al., 2019).

Dieldrin and other environmental toxins have been linked to the development of Parkinson's disease because of their effects on histone H3 and H4 acetylation. Paraquat, another neurotoxic implicated in the development of Parkinson's disease, is likewise connected with elevated levels of acetyl coenzyme A. (Feng et al., 2015).

Low levels of lysine 4 trimethylation in histone H3 are also associated with the decreased dopamine levels found in PD patients (H3K4me3). Hypomethylation of the TNF gene was found to be the underlying cause of susceptibility in the substantia nigra region of the brain. Overexpression of TNF causes neuronal death in the Parkinson's disease brain (Renani et al., 2019).

Numerous other genes involved in Parkinson's disease are likewise regulated by histone changes. Neuronal cells undergo phosphorylation, which enhances histone deacetylation when PINK1 interacts with the transcriptional repressor HDAC3. Furthermore, synuclein dramatically suppresses PARP1 protein by kicking off nitric oxide synthase and generating nitric oxide (Rathore et al., 2021; Renani et al., 2019).

4.2.4 Modifying PD using Non-Coding RNAs

Dopaminergic neurons were found to be miR133b's target, suggesting that miR133b affects the development and activity of these midbrain neurons. This miRNA was shown to be dramatically downregulated in PD patients' midbrain tissue (Renani et al., 2019).

The midbrain miRNA miR132 is also associated with the development of dopaminergic neurons. Increasing miR132 expression reduces synthesis of the target protein, nuclear receptor related protein 1, in an animal model of Parkinson's disease (Nurr1). In the neuroendocrine control of the hypothalamic-pituitary-adrenal axis, the nuclear receptor transcription factor Nurr1 is pivotal. Recent research indicates that Nurr1 is essential for the maturation, migration, and survival of dopaminergic neurons, and this protein is widely distributed throughout the brain, but is most abundant in the substantia nigra (Renani et al., 2019).

Synuclein expression can also be controlled by certain microRNAs. Neurons have been shown to express 40 times more miR7 than other cell types. By binding to synuclein mRNA, miR7 decreases the amount of synuclein protein in the body. In addition, miR153 and miR7 can regulate -synuclein production via 3′UTR and suppress mRNA-related activity. It has also been established that miR-106a causes elevated amounts of synuclein protein (Renani et al., 2019).

A decrease in the transcription of two crucial PD genes, PARK2 and PARK7, has been linked to a reduction in miR34b and miR34c in the brain cells of PD patients (Renani et al., 2019).

The microRNA miR205 has been identified as a key regulator of the LRRK2 gene, and previous studies have shown that people with PD had significantly lower miR205 levels in the corpus striatum and frontal brain. LRRK2 gene expression is upregulated in response to miR205 suppression. In addition, miR205 has therapeutic implications, as it has been demonstrated to reduce LRRK2 gene expression. MiR-10a, 10b, 212, 132, and 495 levels were

also observed to be different in PD patients compared to controls in a number of investigations (Renani et al., 2019).

4.3 Epigenetic Dysregulation in Huntington's Disease

4.3.1 The Huntington's Disease Pathogenesis

Increased numbers of cytosine-adenine-guanine (CAG) repeats in the Huntingtin (HTT) gene's coding region are responsible for the neurological illness known as Huntington's disease, which is inherited in an autosomal dominant fashion. CAG repeat lengths longer than 40 cause HD. The general consensus is that CAG repeat lengths under 35 are benign, and that CAG repeat lengths between 36 and 39 show incomplete penetrance. Polyglutamine-expanded mutant Htt (mHtt) and N-terminal portions of Htt are predominantly found to form insoluble protein aggregates in the nucleus, in contrast to the normal Htt protein, which is predominantly located in the cytoplasm. Even though Htt/mHtt is widely distributed in the central nervous system and the rest of the body, its neuropathological consequences are most pronounced in the striatum. In the striatum, neuronal death happens in several regions as the disease advances. Most distinguishing among HD's movement symptoms is chorea, which is characterized by abnormal, involuntary movements that manifest in a variety of ways across the body. In many cases, the severity of the sickness is exacerbated by the presence of accompanying psychological problems (Thomas, 2019).

4.3.2 Methylation of DNA in Huntington's Disease

The effects of mutant HTT expression level on DNA methylation were studied in the striatal cells of a mouse model of HD, where it was discovered that the majority of regions were hypomethylated. The genes with altered methylation have been associated with neurogenesis, neurodifferentiation, and neuronal signaling (Lardenoije et al., 2018).

Tissue from the prefrontal and parietal brain of HD patients showed an elevated amount of global methylation compared to that of control group participants. Full-length huntingtin (HTT)-expressing mouse striatal cells in culture show that promoter methylation leads to downregulation of several genes involved in regulating neuronal growth, migration, and cell signaling (Bertogliat et al., 2020).

Transcriptional failure has been associated to HD etiology. The presence of mHTT has been shown to alter the methylation of transcriptional regulators in regions of the genome with low CpG content, as shown by a genome-wide research in cell lines generated from mouse striatal neurons (Mohd Murshid et al., 2020). Ap-1, Sox2, Pax6, and Nes genes are transcriptional regulators whose expression levels are drastically lowered due to extensive methylation of their promoter regions. Given the essential role these genes play in brain development, DNA methylation-mediated reduction of hippocampal neurogenesis may be a primary cause of cognitive decline in HD (Lardenoije et al., 2018).

The pathophysiology of disease in the brain's cerebral cortex has been linked to global hypermethylation. It has been shown that 5-hmC levels are decreased throughout the entire genome in a mouse model of HD, especially in the striatum and the cerebral cortex (Bertogliat et al., 2020).

4.3.3 HD and the Role of Histone Modification

Hypoacetylation of histones and altered gene expression in the in vivo and in vitro model of HD were predicted to arise from mHtt binding to the histone acetyltransferase domain of the CREB-binding protein (Thomas, 2019).

More so, widespread reductions in acetylated histone H3 were observed in HD animal models with poor cognitive performance. Hypoacetylation on histones H3 and H4 was linked to the animal model, and it was observed that histone acetylation was decreased at specific gene loci,

most notably the promoter regions of genes known to have downregulated in HD, such as Penk1, Drd2, Grin1, and Actb. Several genome-wide investigations have pinpointed multiple hypoacetylated locations in the striatum of animal models, with a focus on histone 3 at lysines 9 and 14 (H3K9, H3K14) (Thomas, 2019).

The brain tissue of HD patients has been reported to have an aberrant phosphorylation level of histone H3. Primary striatal neurons transfected with a mutant version of the HTT gene showed decreased levels of histone H3 phosphorylation, especially at serine 10. Abnormalities in histone monoubiquitylation, which are regulated by mHtt, have been connected to the transcriptional dysregulation observed in HD (Thomas, 2019).

4.3.4 Role of HD Non-Coding RNAs

In a typical situation, HTT is thought to inhibit nuclear translocation of the repressor element 1 silencing transcription factor (REST) by interacting with it. However, mutant HTT cannot, leading to elevated levels of nuclear REST. A rise in nuclear REST levels causes all sorts of havoc in gene expression because REST controls so many genes, including many miRNAs. Neuron-specific miR-132 expression was shown to be reduced in HD patients, but miR-29a and miR-330 levels were increased, all among the miRNAs impacted by REST. In addition, miR-132, miR-22, miR-29c, miR-128, miR-138, miR-218, miR-222, miR-344, and miR-674 were all downregulated in two animal models of HD (Lardenoije et al., 2018). The mutant HTT (mHTT) has been shown to suppress miR-125b, miR-146a, and miR-150 production in STHdhQ111/HdhQ111 cells in vitro. However, the suppression of miR-146a is a result of a higher level of cellular tumor antigen p53, which is the target of miR-125b and miR-150. A decrease in miR-146a expression is caused by an increase in p53 due to p65-mediated transcription factor repression. Increased HTT levels, mitochondrial dysfunction, and neurodegeneration have all been linked to elevated p53 levels due to the presence of p53-responsive components in the HTT gene (Lardenoije et al., 2018).

Chapter 5: Therapeutic Interventions Based on Epigenetics in Neurodegenerative Disorders

5.1 Targeting Epigenetic Alterations with Epidrugs

This epigenetic plasticity provides a compelling case for investigating reversal in epigenome by therapeutic interventions as a means of ameliorating disease characteristics (Ganesan et al., 2019). Dysregulation of epigenetic enzymes has been linked to a wide range of disorders because of the wide range of cellular and metabolic processes they regulate. Since epigenetic enzymes permit reversible alterations, modulating their activity may be a useful therapeutic strategy for treating some diseases (Singh et al., 2018). Epigenetic medicines, also known as epidrugs, are pharmaceuticals that modify epigenetic marks on DNA and histones by modulating the activity of enzymes involved in epigenome modification (Miranda Furtado et al., 2019).

In order to cure a wide variety of illnesses, the Food and Drug Administration (FDA) of the United States has approved multiple medications based on epigenetics (Miranda Furtado et al., 2019). Decitabine and azacitidine, two cytidine-related chemicals, were first approved for the treatment of myelodysplastic syndrome. These two drugs work by inhibiting the DNA methyltransferase enzyme. Both hydralazine and procainamide, used to treat hypertension and heart rhythm disorders, were also given the green light. Histone deacetylase inhibitors (HDACi) vorinostat and romidepsin have been approved by the US Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma, while panobinostat and belinostat have been approved for the treatment of peripheral T-cell lymphoma and multiple myeloma, respectively. The therapeutic promise of the "first generation" of epigenetic medicines now used in clinical practice lies with DNMTi and HDACi substances. The development of histone acetyltransferases (HATs), histone methyltransferases (HMTs),

histone acetyl-binding proteins (PAHs), and histone methyl-binding proteins (PMHs) are all examples of epigenetic enzymes and associated proteins that have been targeted by the 33 so-called "second generation" epigenetic drugs (Y. Qi et al., 2016). In addition, the pharmacological action and cytotoxicity of newly produced medications are continually assessed in order to gain insight into their activity in preclinical and clinical research and ultimately create a therapy for epigenetics that is both safe and effective (Miranda Furtado et al., 2019). Table 1 and Figure 6 show some of the epigenetic medicines that target specific enzymes.

Table 1: List of different categories of epigenetic drugs (Adapted from Berdasco & Esteller, 2019; Y. Qi et al., 2016; Singh et al., 2018).

Category	**Epigenetic Drugs**
DNMTi	Decitabine, Azacytidine, Procainamide, Procaine, Epigallocatechin-3- gallate (EGCG), Zebularine, Hydralazine, Guadecitabine, 4′-thio-2′- deoxycytidine (TdCyd)
HATi	Curcumin, C-646, PU-139, Anacardic Acid, Garcinol
HDACi	Trichostatin A, Vorinostat, Tefinostat, Givinostat, Panobinostat, Chidamide, Belinostat, Entinostat, Romidepsin, Valproic acid, Plitidepsin, Tasquinimod, Sodium butyrate, CG-1521, SB939, CHR-2845, CHR-3996, CUDC-907, ACY 241, AR-42, KA2507, MPT0G009, ORY-2001, 4-phenylbutyrate, Pivanex, Resveratrol, Abexinostat, Resminostat, Dacinostat, Droxinostat, Tubastatin A, Abexinostat, Mocetinostat, Givinostat, Quisinostat, Pracinostat

HDMi	Pargyline, Clorgyline, Bizine, GSK2879552, GSK-J4, KDM5-C70, JIB-HDMi 04, EPT-103182, Tranylcypromine. Phenelzine, INCB059872, ORY- 1001, IMG-7289, ORY-2001
HMTi	Allantodapsone, BIX-01294, EPZ-6438, GSK126, GSK3235025, GSK332659, CPI360, DZNep, GSK343, EI1, UNC0638, EPZ004777, UNC0224, Tazemetostat, Ellagic acid, MAK683, Pinometostat, DS-3201b, GSK2816126, GSK3326595, CPI-1205

5.1.1 Inhibitors of DNA Methyl transferases (DNMTi)

The DNMTs enzyme family includes DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L, and it regulates DNA methylation. Cancer, genetic mutations, neurological and immune system problems have all been associated with changes in DNA methylation (such as hypermethylation and hypomethylation). As a result, DNMTs have emerged as a promising therapeutic target, and various small-molecule inhibitors have been developed that act specifically on the DNMT family. Nucleoside cytidine chemotype DNMT inhibitors 5-aza-cytidine (Azacitidine or Vidaza) and 5-aza-2-deoxycytidine (Decitabine or Dacogen) have been approved by the FDA for the treatment of myelodysplastic syndrome. These nucleoside analogs reduce DNA methylation by binding to the chromatin and forming an irreversible complex with the DNMTs, which leads to the degradation of the DNMT enzymes. Hydralazine, procainamide, epigallocatechin 3-gallate (EGCG), SGI-110, and RG108 are only some of the key non-nucleoside analogs that target DNMT that are now under investigation. In contrast to

DNMTs, which are integrated into DNA, inhibitors from non-nucleoside oligonucleotides engage with DNMTs directly, either at the 3′ UTR, resulting in transcriptional suppression, or at the enzyme active site, reducing their enzymatic activities (Prachayasittikul et al., 2017; Singh et al., 2018).

5.1.2 Anti-histone acetyltransferase agents (HATi)

Although histone acetyltransferases (HATs) were first recognized as tumor suppressor regulators, they have now been linked to the development of cancer, viral infections, and even some lung ailments. In the N-terminal tails of histone proteins, there may be several basic lysine and arginine residues. Enzymes called histone acetyltransferases (HAT) use acyl coenzyme A donors to convert lysine residues into the corresponding acyl-lysine residue. About 20 HATs have been identified in humans, including both nuclear and cytoplasmic localizations. Lysine acetylation, which is linked to an increase in the size of the side chain, is the primary process connected with HATs, and it occurs not only in histones but also in non-histone proteins in the nucleus and at other cellular locations. First, molecules must be chosen that feature 35 the lysine substrate and acetyl-coA complex produced in the active site of those enzymes during acetylation processes. The acetyl-CoA cofactor was bonded to a lysine in the histone peptide substrate using covalent chemistry to accomplish this. A number of naturally occurring substances, including curcumin, anacardic acid, and garcinol, have been shown to suppress HAT (Ganesan, 2018; Prachayasittikul et al., 2017; Singh et al., 2018).

The enzymes sirtuins and zinc-dependent histone deacetylases (HDACs) work together to reverse lysine acylation, which is what the Histone Deacetylase Inhibitors (HDACi) do (Sirts). In biology, HDACs and sirtuins are responsible for reverting acyl-lysine residues to protonated lysine. This results in the nucleosome becoming compacted, which in turn silences genes. The reactivation of latent pathways in DNA repair, immunomodulation, tumor suppression, and apoptosis is a major factor in ensuring that such enzymes are inhibited, and therefore

reprogramming is successful. Around 18 HDACs have been identified in various taxa; these have been further subdivided into four distinct groups based on their structural and phylogenetic similarities and differences. Several biological processes, including differentiation, proliferation, apoptosis, and senescence, rely on the actions of these enzymes (Ganesan, 2018; Prachayasittikul et al., 2017; Singh et al., 2018).

Some HDACi chemotypes are based on short chain fatty acids (such valproic acid, sodium butyrate, phenylbutyrate, and pivanex), while others use cyclic tetrapeptides and natural chemicals, bicyclic depsipeptides (like romidepsin), or selective classes consisting of hydroxamic acids (e.g., vorinostat, panobinostat, belinostat, and dacinostat). Hydroxamic acid analogs make up the bulk of the drugs in Phase 3 clinical studies at present. Vorinostat, a medicine licensed by the Food and Drug Administration, has been found to enhance the clinical efficacy of hydroxamic analogs (Prachayasittikul et al., 2017; Singh et al., 2018).

Modification of histone methylation is thought to be crucial in epigenetic control and has substantial therapeutic potential for diseases including cancer and genetic abnormalities, which may be treated with inhibitors of enzymes called histone demethylases (HDMi). There is an enzyme family called lysine demethylases (KDMs) whose job it is to oxidatively remove methyl groups from lysine residues. KDM demethylases can be broken down into two groups, the LSDs and the Jumonji C demethylases, each of which performs a unique role. As a result of their reliance on the enzyme flavin adenine dinucleotide mutase, humans have two LSD isoforms: LSD1 (KDM1A) and LSD2 (KDM1B) (FAD). Inhibitors of amine oxidases, such as tranylcypromine, phenelzine, and pargyline, also block the activity of the HDM KDM1A because of their structural and sequence similarity to the LSD1/KDM1 demethylases (Ganesan, 2018; Prachayasittikul et al., 2017; Singh et al., 2018).

5.1.3 Antagonizers of Histone Methyl transferases (HMTi)

Methyl groups are transferred from SAM to the lysine or arginine side chains of the target protein by enzymes known as histone/protein methyl transferases (HMTs/PMTs). Enzymes that bind SAM and amino acids are good targets for small-molecule inhibitors due to structural features of the binding pocket that enhance inhibitor engagement. It's well established that these tiny molecules exert their action by occupying enzyme binding sites traditionally occupied by substrates or the cofactor SAM (Prachayasittikul et al., 2017; Singh et al., 2018).

SAM-competitive inhibitors, such as BIX-01294, BIX-01338, GSK126, and Tazemetostat, are used for epigenetic therapy. In addition, various novel SAM-competitive inhibitors are found and, for the treatment of hematological malignancies, are currently being tested in clinical trials. These include EPZ6438, GSK2816126, and CPI-1205 (Prachayasittikul et al., 2017; Singh et al., 2018).

Different epigenetic medicines' chemical structures are shown in Figure 6. (Adapted from Prachayasittikul et al., 2017). Targeting specific epigenetic processes and thereby affecting subsequent gene transcription is the goal of epigenetic drugs like DNA methyltransferase inhibitors (DNMTi), histone demethylase inhibitors (HDMi), histone methyltransferase inhibitors (HMTi), histone acetyltransferase inhibitors (HATi), and histone deacetylase inhibitors (HDACi).

5.2 Using Epigenetics to Treat Alzheimer's

Donatiezil, rivastigmine, galantamine, and Huperzine A are cholinesterase inhibitors that are used as a first line of treatment for Alzheimer's disease (AD) because they boost acetylcholine and so improve memory and cognitive impairment. Neuronal dysfunction can be treated with namzaric and memantine, which work by reducing excessive amounts of the neurotransmitter glutamate (Solanki et al., 2016).

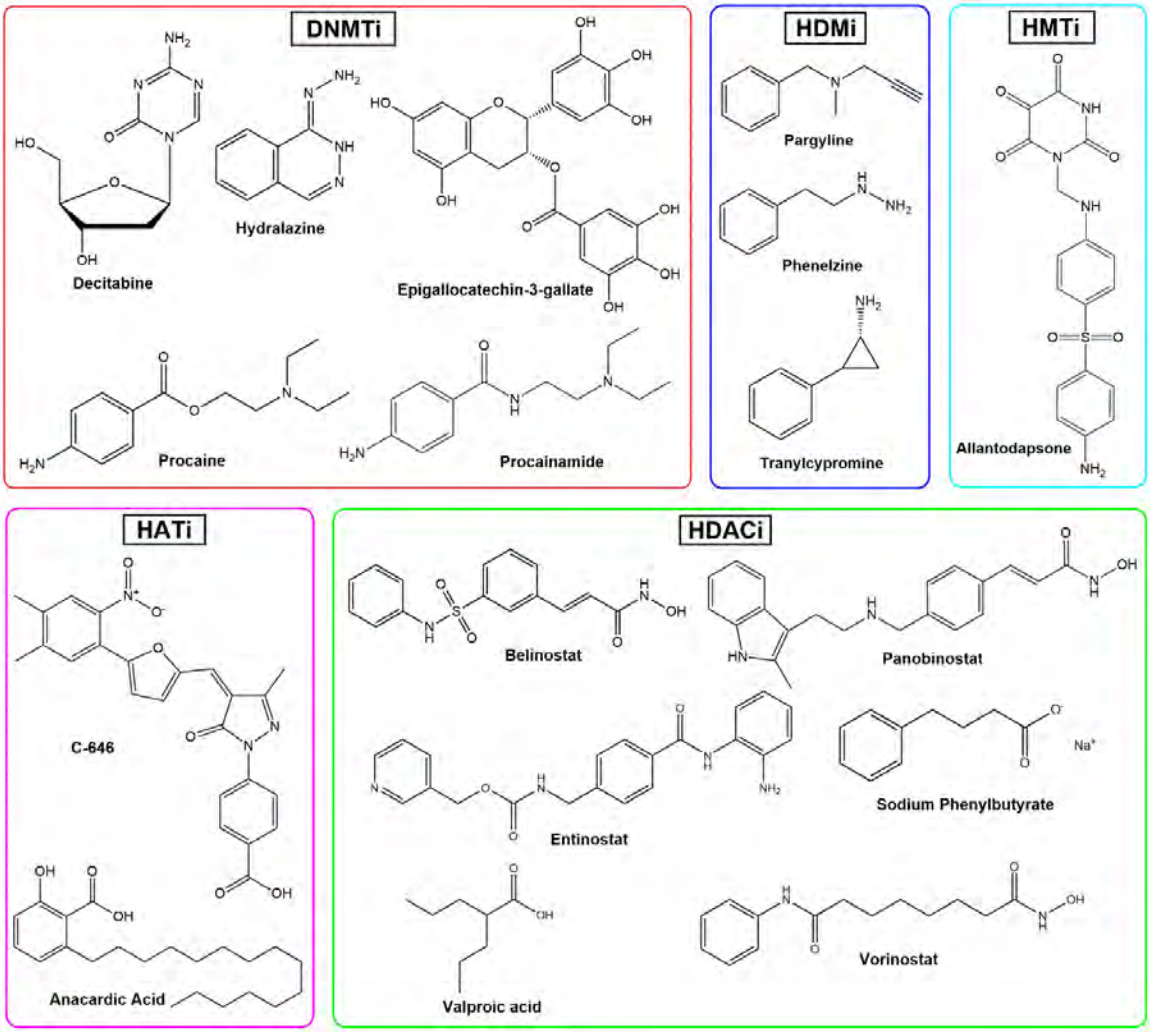

Figure 6: Chemical structure of different epigenetic drugs (Adapted from Prachayasittikul et al., 2017). Epigenetic drug consisting of DNA methyltransferase inhibitors (DNMTi), histone demethylase inhibitors (HDMi) histone methyltransferase inhibitors (HMTi), histone acetyltransferase inhibitors (HATi) and histone deacetylase inhibitors (HDACi) target specific epigenetic processes and influence the subsequent gene transcription.

Furthermore, numerous medications targeting epigenetic machinery are in the development stage with substantial clinical benefits.

The overproduction of A protein is caused by hypomethylation of the genes encoding for A protein. Restoration of Dnmt3a2 DNA methyltransferase, which is depleted in the hippocampus of the animal model, rescues memory and learning. Betaine, a methyl donor, has been shown to ameliorate AD-related memory loss. Another methyl donating molecule, S-adenosylmethionine (SAM), is shown to be low in cerebral fluid samples from AD patients.

By elevating the expression of PSEN1 and BACE1, SAM reduces the production of A and tau phosphorylation and, in turn, enhances cognitive performance. Hypermethylation of TXNRD1 and NUDT15, genes associated with enhanced cognitive function in AD patients, is increased by vitamin B12 administration. Another methyl donor that boosts patients' brainpower is folic acid. The use of DNA methyltransferase or methyl donor as a treatment for Alzheimer's disease (AD) would thus be feasible (Xiao et al., 2020).

Reducing methylation levels in particular genes is a potential treatment method because of its association with the etiology of AD. Inhibitors of DNMT are currently used to treat hematological cancers. Some other neurodegenerative illnesses, like Friedreich's ataxia, are also being treated with DNMT inhibitors. Though DNMT inhibitors show promise as a treatment for Alzheimer's disease, they cannot yet be administered to patients due to concerns over their lack of gene specificity and safety. It has been discovered that UNC0642, a powerful pharmacologic inhibitor of the methyltransferase activity of G9a/GLP, binds to histone H3 Lys 9 (H3-K9), lowering 5mC and raising 5hmC, and thereby restoring cognitive function. By reducing brain methylation levels and promoting the oxidation of 5mC to 5hmC, suppressing the Tet1 gene that is involved in DNA methylation improves cognitive performance (Xiao et al., 2020).

Histone deacetylases (HDACs) inhibit transcription by blocking access to transcription factors, and they have been linked to memory loss and learning difficulties. HDAC inhibitors (HDACi) are currently under investigation as a potential treatment for Alzheimer's disease. Improvements in contextual freezing abilities have been observed in patients with Alzheimer's disease after treatment with the HDACi trichostatin A. The initial HDACi identified, valproic acid has been validated for its usefulness in memory enhancement. Inhibitors of histone deacetylase 2 (HDAC2) have been found to improve memory by promoting dendritic spine formation and maturation. By stimulating neurotrophin production via the protein kinase C

(PKC)-cyclic AMP response element-binding protein (CREB) pathway, sodium phenylbutyrate, another HDACi, also enhances memory. HDACi, such as M344, protect against cognitive decline by lowering A expression and modulating many pathogenic pathways. Decreased Alevels and enhanced memory performance can be achieved by using either hydroxamide, a class I and II HDACi, or mercaptoacetamide, a class II HDACi. One genome-wide analysis found an inverse relationship between low PU.1 expression and Alzheimer's disease risk. Vorinostat, an HDACi, has been shown to decrease PU.1 expression and may offer hope as a therapy for AD in a high-throughput review of FDA-approved medicines. Further evidence for the significance of HDACi in AD patients comes from studies examining the effects of the specific HDAC3 inhibitor RGFP-966 on cognitive performance and A and tau production in neurons. Therefore, HDACi may provide a viable pharmacological therapy option for Alzheimer's disease (Xiao et al., 2020).

The memory-related protein CREB binding protein (CBP) cannot be produced without the help of histone acetyltransferase (HAT). Memory problems in an Alzheimer's disease mouse model may be recoverable if CBP is expressed. Methyltransferases of histones Hypermethylation of histone proteins is associated with cognitive decline and synapse dysfunction in Alzheimer's disease (AD), however inhibitors can reverse these effects (Xiao et al., 2020).

The pathogenesis of Alzheimer's disease is heavily influenced by noncoding RNAs. Reducing the expression of amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) by RNA interference techniques is a promising therapeutic approach. Anti-microRNAs and analogues of microRNAs have been created to block the translation of genes involved in Alzheimer's disease. APP and BACE1 expression is lowered by miR-384 mimics, suggesting that miR-384 is a significant target in AD. To keep things in check, anti-microRNAs reduce the abundance of their matched counterparts. Anti-microRNA-146a medication improves cognitive function and suppresses inflammation, while microRNA-146a is

upregulated in the AD mouse model. In addition, many distinct microRNAs hold promise as therapeutic avenues for Alzheimer's disease. Studies in animals have shown that inhibiting microRNA-34c, which is abundantly expressed in the hippocampus in Alzheimer's disease, enhances memory performance. The lncRNA BACE1-AS is associated with BACE1 protein expression, and its knockdown by small interfering RNA enhances memory and learning in AD models of the disease in animals. The expression of the BACE1 gene is suppressed in vitro by miR-29c, miR-124, and miR-339-5p. Hippocampal neurons were used in an in vitro study, and it was found that microRNA-101 inhibited A and APP expression. MicroRNA-153 inhibits APP expression in an AD cell-free model. Therefore, non-coding RNAs may one day serve as therapeutic targets for Alzheimer's disease treatment (Xiao et al., 2020).

Until now, A and tau protein have been the primary targets of clinical research for Alzheimer's disease. Multiple clinical studies are currently looking into the potential of epigenetic intervention in the treatment of Alzheimer's disease. Eight individuals with AD were given oral betaine; however, the effectiveness of betaine could not be determined due to a lack of a control group and a small sample size. There is evidence that supplementation with S-adenosylmethionine and other nutriceuticals can improve neuropsychiatric functions in AD patients by about 30% relative to healthy controls. RDN-929, a selective HDACi, has been studied in a phase-I clinical trial for the treatment of Alzheimer's disease. Clinical trials for EVP-0334, also known as FRM-0334, an HDACi with CNS-penetrating properties, have concluded in phase I. Growing data suggests that epigenetics plays a crucial role in AD, suggesting that treatments focusing on epigenetics may represent significant advances in the management of AD (Xiao et al., 2020).

5.3 Using Epigenetics to Treat Parkinson's

To date, levodopa and a handful of dopamine agonists like tolcapone, ropinirole, and apomorphine hydrochloride have proven effective in treating Parkinson's disease (Solanki et al., 2016). There are, however, two significant epigenetic therapeutics available now, through DNA methyltransferase inhibitors and histone deacetylase inhibitors.

Reduced DNA methylation has been linked to cognitive impairment, and abnormal SAM metabolism has been identified as a hallmark of PD. Folate, vitamin B12, methionine, and choline supplementation may be used to replenish SAM levels and hence reverse this decline. The expression of neuroprotective genes like tyrosine hydroxylase and the transcription of PD pathogenic genes like synuclein and UCHL1 could be regulated by DNMT inhibitors like 5'-aza-2'-deoxycytidine (5'-aza-dC) (Renani et al., 2019).

By reducing the amount of deacetylated histones, histone deacetylase inhibitors can increase chromatin expansion, activate a variety of neuronal growth factors, and shield neurons from inflammation. Neurotrophic factors like BDNF, HAP70, GDNF, synuclein, p21, and Gelosolin are all influenced by histone deacetylase inhibitors (Renani et al., 2019).

Both vorinostat and sodium butyrate can inhibit histone deacetylase and increase acetylation of histones H3 and H4 in a PD animal model, suggesting that these compounds may be useful in the treatment of PD. Sodium butyrate is able to rapidly cross the blood-brain barrier (BBB) and inhibits Class I and Class II histone deacetylases. Sodium butyrate has been shown in a number of studies to be neuroprotective by increasing the synthesis of anti-inflammatory molecules including heat shock protein 70 (HSP70). Many lysines on histones H2A and H2B, lysine 9 on histone H3, and lysine 8 on histone H4 are acetylated in human dopaminergic neurons when sodium butyrate is present (Renani et al., 2019).

Urea cycle disorders were the first medical conditions for which phenylbutyrate was licensed as a treatment. Adding a phenyl group to the sodium butyrate carbon at position 4 produces phenylbutyrate. This compound, like sodium butyrate, inhibits histone deacetylase and has shown considerable success in delaying the death of dopaminergic cells in PD animal models. In addition, phenylbutyrate can increase DJ1 protein expression, which in turn protects dopaminergic neurons from oxidative damage and -synuclein neurotoxicity. In this way, phenylbutyrate can stop the buildup of alpha synuclein, which causes motor symptoms and cognitive decline (Renani et al., 2019).

As a hydroxamate-based histone deacetylase, vorinostat can increase the production of the neuroprotective proteins HSP70 and BCL2 in PD animal models. Neuroprotective BDNF and GDNF factors are released from astrocytes after treatment with vorinostat, which has been demonstrated to increase histone hyperacetylation. Trichostatin A, derived from the bacterium Streptomyces hygroscopicus, is another, less popular medication. Initially identified as an antifungal antibiotic, its histone deacetylase inhibitory action was first reported in 1990. (Renani et al., 2019).

By blocking the generation of proinflammatory cytokines by activated microglial cells, trichostatin A protects dopaminergic neurons from dying in Parkinson's disease. Furthermore, trichostatin A protects neurons from apoptosis and mitochondrial dysfunction (Renani et al., 2019).

Apicidin is an inhibitor of histone deacetylases 2 and 3, with poorer selectivity for histone deacetylase 8. Apicidin is derived from the cyclic tetrapeptide analog. Apicidin raises the expression of the HSP70 chaperone, which in turn increases the degree of histone acetylation and, consequently, increases neuroprotection. Histone deacetylases 2 and 3 are the most abundant histone deacetylases expressed in the substantia nigra region of the brain. In an animal

model of PD, the deacetylase sirtuin 2 suppresses alpha-synuclein and decreases the toxicity it causes (Renani et al., 2019).

By blocking the activity of histone deacetylases, valproic acid (2-propylpentanoic acid) has beneficial effects on the brain. Valproic acid increases histone acetylation and decreases inflammatory protein levels, but it also increases the expression of BDNF and GDNF, two neuroprotective factors. For the treatment of Parkinson's disease (PD), the histone deacetylase inhibitor valproate has shown the most promise (Renani et al., 2019).

It's possible that monoamine oxidase inhibitors like selegiline and rasagaline aid in reestablishing normal levels of lysine 4 methylation in histone H3 (Renani et al., 2019).

5.4 Huntington's Disease and Epigenetic Treatment

The only medicine approved by the Food and Drug Administration (FDA) for treating Huntington's Disease symptoms, such as involuntary writhing motions, is tetrabenzine (Solanki et al., 2016). However, various epigenetic based treatments targeting the unique pathology of Huntington's Disease are currently in the development stage. Drugs that block methyl transferases may be useful in the treatment of HD because they may prevent histone acetylation from occurring, which is a key step in the disease's progression. Anthracyclines analogs, such as chromomycin and mithramycin A, are a class of antibacterial bacterial medicines with anticancer properties that can modify histone methylation indirectly by binding to the GC. Both chromomycin and mithramycin have been shown to reduce the capacity of transcriptional activators to bind to the CpG-rich gene promoter, hence preventing the transcription of genes that trigger oxidative damage and death. Mithramycin treatment prolongs life, enhances motor function, and considerably delays neuropathological complications in a mouse model of HD. Improving survival and neuroprotection in an HD mouse model after mithramycin treatment

suggests that repressed expression of genes necessary for neuronal growth and function may be restored as a result of the intervention (Thomas, 2019; F. Wang et al., 2014).

HD treatment also focuses on modifying DNA by means of hydroxymethylation. The total level of DNA hydroxymethylation is much lower in the cortex and striatum regions in the HD mouse model compared to the control group, suggesting that increasing DNA hydroxymethylation could prevent HD symptoms and represent a promising therapeutic target for the treatment of HD. In recent years, evidence has accumulated showing that certain chemicals can influence global DNA hydroxymethylation. Dimethyl sulfoxide has been shown to greatly increase both global and localized DNA hydroxymethylation in an in vitro model. This could result in upregulation of genes involved in DNA hydroxymethylation, including TET and nucleotide excision repair (GADD45), and downregulation of genes involved in DNA methylation, like Dnmt1 and Dnmt3b, through inhibition of their transcription. It has been shown that vitamin C can improve the catalytic efficiency of TET dioxygenases in oxidizing 5mC directly, suggesting that it may also serve as a cofactor component for TET enzymes. Vitamin C causes a worldwide decrease in 5mC levels in mouse embryonic stem cells by increasing the oxidation products of 5mC, particularly 5-carboxylcytosine and 5-formylcytosine. Since DNA hydroxymethylation is drastically decreased in the cortex and striatum of HD mice brains, it stands to reason that medications designed to increase DNA hydroxymethylation may have therapeutic value. Additional studies are needed to assess the medicinal potential and clinical efficacy of these compounds (F. Wang et al., 2014).

The DNA conformations are loosened by HDAC inhibitors, which might have a systemic effect on gene activation or repression. Suberoylanilide hydroxamic acid and sodium butyrate are the first medicines to show promise in suppressing HDAC in an HD animal model. Thanks to these pivotal discoveries, clinical trials with HDAC inhibitors in HD patients have begun. Improving memory in HD mice with gene knockout, sodium butyrate, or suberoylanilide hydroxamic acid

treatment. Polyglutamine has been shown to decrease histone acetylation in in vitro HD models, and HDAC inhibitors have been shown to mitigate the toxicity caused by polyglutamine. When compared to other kinds of HDAC inhibitors, sodium butyrates are at the forefront of clinical development due to their high bioavailability in the central nervous system (CNS). Studies on animals and humans have demonstrated that sodium butyrate is safe and well-tolerated. Sodium butyrate dramatically increases survival in a mouse model of cancer by modifying histone alterations, enhancing motor performance, and decreasing neuropathologic consequences. Since phenylbutyrate has FDA approval and sufficient data on its pharmacology, pharmacokinetics, toxicity, and dose, it may be worth looking into. Phenylbutyrate alters chromatin structure and ameliorates motor deficits and neuropathological traits in HD animal models. Clinical trials involving HDAC inhibitors have been conducted on HD patients because of their potential to improve overall neuropathology) and mortality in HD animal models. Histone deacetylase inhibitor 4b (HDACi 4b) treatment dramatically reduced histone H3 acetylation and increased mRNA expression in a mouse model of HD. When HDACi 4b was administered to transgenic mice, not only were their brains brought back to normal, but so were their bodies and their motor abilities. HDACi 4b treatment specifically altered pathways that code for cellular proteins that undergo post-translational modifications including phosphorylation and ubiquitination. Through blocking kappaB kinase with HDACi 4b, the Htt protein is deacetylated, phosphorylated, and ultimately degraded via the ubiquitin-proteasomal and autophagic pathways. It has been shown through research that inhibiting histone deacetylases (HDACs) HDAC3 and HDAC1 can ameliorate mhtt-induced metabolic problems and ameliorate mhtt-induced neurotoxicity. Two highly effective HDAC3 inhibitors, HDACi 4b and 136, were shown to be the most effective in reversing the expression of several Huntington's disease-related genes (HD). These researches show that HDACi 4b is effective and has significant biological effects, suggesting it could be a great therapy alternative for HD

patients. Taken as a whole, these studies demonstrate that HDAC inhibitors can improve symptoms by repressing prodeath genes that are elevated in HD and repressing survival genes that have been suppressed in HD. Furthermore, more research is needed to determine the precise processes through which HDAC inhibitors alter neuronal activity (Lee et al., 2013)

Given that HD is caused by mutations in htt's exon 1, genetic engineering to silence mhtt expression via ncRNAs has been proposed and is currently under investigation as a potential treatment. While the wild-type htt protein is crucial to neuronal function, the mutant form is neurotoxic. Since most HD patients have one mutant heterozygous htt allele and one regular-type htt allele at the location, eradicating the mutant allele while keeping the regular allele's expression intact would be a significant hurdle for gene therapy. Therefore, it may be possible to selectively suppress mutant htt by the development of noncoding short RNAs as nucleic acid therapy. Motor deficits and neuropathological traits are ameliorated by RNA interference using adeno-associated virus-small hairpin RNA (shRNA) in a transgenic mouse model of HD. Most studies of RNA interference using adeno-associated virus-miRNA, adenovirus-shRNA, lentivirus-shRNA, and cholesterol-conjugated siRNA show that reducing the aggregating mhtt improves motor performance and decreases neuropathological deficits. Compared to unmodified RNA, single-stranded siRNAs (ss-siRNAs) are both more efficient (100-fold) and more selective (30-fold) at suppressing the production of the mhtt allele. The expression of the mutant htt allele is reduced in an HD animal model by intraventricular infusion of ss-siRNA (Lee et al., 2013).

Chapter 6: Future Prospects and Areas of Discussion

Beginning with brain development, epigenetic regulations significantly control the expression of specific genes at specific times, which in turn is directly associated with the formation of neuroplasticity, concurrent neurogenesis, progressive neurodegeneration, and transmission dependent cognitive processes. Neuronal structure and function progressively deteriorate, leading to neuronal death, as typified by neurodegenerative illnesses. Function can be impaired in a variety of ways, and these impairments might worsen as neurodegeneration spreads through the brain. Neurodegenerative illnesses often vary in their underlying pathophysiology and in where the degenerative processes occur. The most common forms of neurodegeneration studied are Alzheimer's disease, Parkinson's disease, and Huntington's disease, but amyotrophic lateral sclerosis and prion disorders also exist and are studied often. Although some neurodegenerative diseases, like HD, have a clear genetic basis, others, like sporadic AD and PD, have a considerably more complex basis of genetics and disease development, presumably implicating gene-gene and gene-environment associations, and thus their underlying pathogenesis remains unknown. Many studies have looked for answers when genetics didn't work, and epigenetic mechanisms have emerged as the most promising. Many researchers are looking into epigenetic dysregulations as a possible cause of premature aging and neurological disorders associated with old age. The interaction between genes and the environment causes these dysregulations. Some similar modifications occur that appear to bridge the apparently diverse pathophysiology of neurodegenerative disorders such as AD, PD, and HD, despite the major disparities in epigenetic marks. For instance, a thorough analysis of the demethylation dysregulations revealed that a distinct methylation pattern in genes causes the hereditary propensity to Alzheimer's disease and Parkinson's disease, including APP, BACE, PS1, PS2, APOE for AD and SNCA, PARKIN16 for PD. Furthermore, abnormal histone acetylation is present in all three disorders studied, with genome-wide deacetylation of

histones being observed in Alzheimer's disease and Parkinson's disease. It has been found that both Alzheimer's disease (AD) and Huntington's disease (HD) share common alterations to histone 3, most notably an increase in tri-methylation of H3K9. Finally, evidence from three of the disorders discussed above shows that deregulation of ncRNAs plays a crucial role in the pathogenesis of these conditions. Changes in miR-132 and miR-29 expression are not only indicative of the aforementioned three age-related neurodegenerative disorders but also of the natural aging process itself. A pattern of altered expression for miR-22, miR-26a, and miR-125 is also characteristic of those disorders (Lardenoije et al., 2015; Singh et al., 2018).

However, understanding the connection between abnormal epigenetic alterations and a variety of cognitive problems is challenging. Whether epigenetic alterations are what actually cause the symptoms or whether they are merely a sign of other brain changes is still debatable. It is possible that epigenetic alterations are just one of many functional consequences of altered intracellular signaling, given that epigenetic modifications like DNA methylation and histone acetylation may represent as an adjacent integration process for a number of different signaling pathways and second messenger system. Dysregulation in several aspects of brain biology, including neurotransmitter release, receptor function, and the molecular signaling pathway, might theoretically lead to a lack of epigenetic activity. Consequently, the ability to treat and, in some cases, prevent certain cognitive deficits would be enhanced by ongoing research into determining whether epigenetic alterations are the cause or consequence of these conditions. Even when a changed epigenetic mechanism is recognized to be at the root of a condition, it is not yet clear how this change emerges across the many gene targets and functionally distinct regions of the brain. Therefore, it is crucial to keep assessing how epigenetic markers influence gene expression and function across the entirety of brain systems, in addition to examining the epigenetic markers linked with a disease (Day & Sweatt, 2012).

Drugs used to alter epigenetic pathways present a unique set of difficulties. Selectivity of enzyme isoform, selectivity of lysines and arginines histone substrate versus non-histone substrate, designing dual inhibitor, using combination of epigenetic drug, and the multimeric complexes of epigenetic enzymes that complicated the translation of in-vitro potency to in-vivo efficacy are just some of the challenges that must be overcome before an epigenetic drug can be designed successfully after an epigenetic target for a disease has been identified. Currently existing epigenetic medications, such as histone deacetylase (HDAC) and dimethyltryptamine (DNMT) inhibitors, have a major drawback in that they are not selective for particular brain regions, neuronal subgroups, or genes. Treatment of diseases characterized by changes in epigenetic expression at a small subset of gene sites is hampered by a lack of selectivity. One such example is the reelin gene, which has been linked to schizophrenia when its methylation is abnormal. Aside from reelin, the methylation of many other genes may be lowered by systemic or even region-specific administration of DNMT inhibitors. Similar increases in histone acetylation of unrelated genes would occur if HDAC inhibitors, which can restore hypermethylation in the reelin gene, were administered. Similarly, because DNA methylation and histone changes work together to regulate transcription, altering one mechanism would have complicated effects on other mechanisms. As a result of these difficulties, epigenetic treatments are unlikely to be used in therapeutic settings. Therefore, further study is required to discover and create more specific epigenetic regulators. To create more specific epigenetic regulators, HDAC enzymes could be used as an alternative. Because different HDAC isoforms are expressed at different levels and have different biochemical functions in various brain regions, it may be possible to maximize spatial and genetic specificity by focusing on a single isoform (Day & Sweatt, 2012). One of the trickiest aspects of epigenetic therapy is developing medicines that can penetrate the blood-brain barrier (BBB). In general, molecules with a dalton count below 500 and a hydrogen bond count below 8 can

cross the BBB. In order to get over this restriction, other methods have been developed, such as BBB rupture, intracerebral implantation, and intracerebroventricular injection. The blood-brain barrier (BBB) can be traversed by a wide variety of medications, including those that block DNMT and those that block HDAC. Efficacy, delivery, toxicity, and patient variables are only some of the many other considerations that must be taken into account (Liu et al., 2018). Several medications that primarily target epigenetic pathways have been approved by the FDA as of 2018. (Table 2). Many of these earliest medications are now approved for use in the treatment of cancer, but research into the use of chemicals that target epigenetic alterations in a variety of other disorders is gaining momentum (Figure 7). (Tzika et al., 2018).

Table 2: Disorders is gaining momentum (Figure 7). (Tzika et al., 2018).

FDA Approved Drug	**Commercial Name**	**Company**	**Drug Class**	**Treatment of Approval**	**Year**
5-Azacytidine	Vidaza®	Celgene	DNMTi	Myelodysplastic Syndrome	2004
Decitabine	Dacogen®	Eisai	DNMTi	Myelodysplastic Syndrome	2006
Vorinostat	Zolinza®	Merck	HDACi	Cutaneous T- Cell Lymphoma	2006
Romidepsin	Istodax®	Celgene Corporation	HDACi	Cutaneous T- Cell Lymphoma	2009
Belinostat	Beleodaq®	Spectrum Pharmaceuticals	HDACi	Peripheral T-Cells Lymphoma	2014
Panobinostat	Farydak®	Novartis	HDACi	Multiple Myeloma	2015

Chidamide	Epidaza®	Shenzhen Chipscreen Biosciences	HDACi	Peripheral T-Cells Lymphoma	2016

Table 2: List of FDA-approved epigenetic drugs (Adapted from Berdasco & Esteller, 2019; Graça et al., 2016).

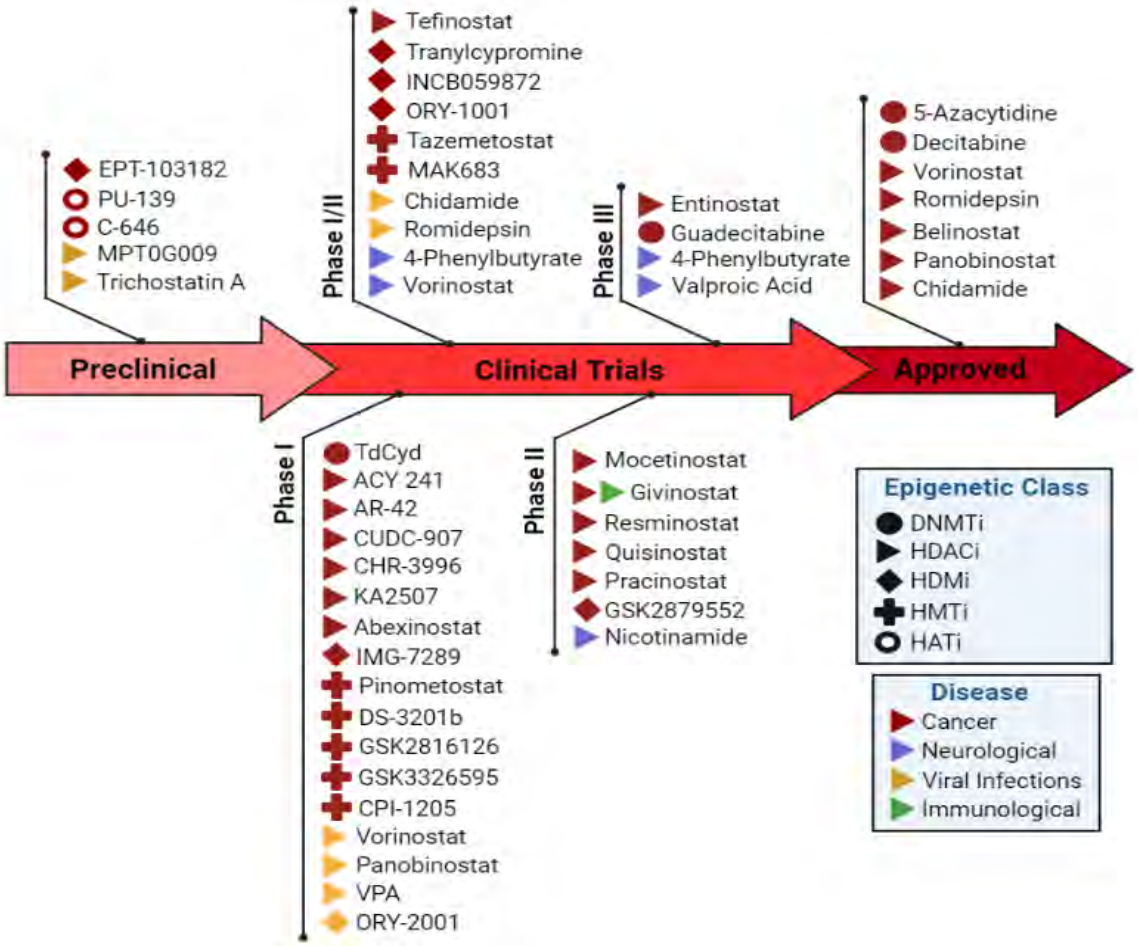

Figure : 7 Drugs targeting epigenetic mechanisms in a variety of human disorders, (Adapted from Berdasco & Esteller et al, 2019).

The Food and Drug Administration (FDA) has licensed several epigenetic medications for the treatment of haematological malignancies, and these drugs are now now being investigated for use in the treatment of neurological, immunological, and viral illnesses. Several kinds of

epidrugs, including histone demethylase (HDMi), histone methyltransferase (HMTi), and histone acetyltransferase (HATi) inhibitors, are currently in preclinical or clinical development for the treatment of a variety of human disorders.

Understanding the functional importance of blocking individual enzymes or their isoforms requires biological techniques in addition to standard chemical medical innovation. In addition to its use in repairing genetic defects, the CRISPR/Cas technique has attracted widespread attention because it may be used to inactivate any gene of interest. Furthermore, CRISPR/Cas could be used to direct effectors toward a particular genomic locus to re-express a gene that has been silenced due to epigenetic suppression, restoring the lost genetic function. More specifically, this is achieved by disrupting the Cas protein's nuclease activity, resulting in inactive Cas (dCas). Single guide RNAs then recruit dCas to the desired chromosomal location, where it can be employed to deliver the fusion protein. The fusion of an enzyme's catalytic site into dCas causes the system to modify the epigenetic signature at that location (epigenetic editing) (Ganesan et al., 2019).

Similarly, to genetic and genomic testing, epigenetic profiling may provide insight that can be used in clinical practice. Genome-wide sequencing, RNA profiling, chromatin immunoprecipitation, and bisulfite conversion are just a few examples of recent advances in high-throughput sequencing technologies that have generated vast amounts of data that could be used to develop a comprehensive understanding of epigenetic dysregulation that is common and disease-specific. These fast accumulating results will unquestionably lay the groundwork for the development of enhanced molecular diagnostics and individualized precision therapeutics, which may include site-specific epigenome editing (Kelly et al., 2010; Qureshi & Mehler, 2014).

However, the high association between epigenetic dysregulation and neurodegenerative disorders suggests that pharmacological medicines targeting epigenetic alteration, such as DNA methylation and histone acetylation, could be beneficial in treating these conditions. Nonetheless, therapeutic interventions targeting these epigenetic dysregulations, especially in the early stage of the diseases, appear to be a potential therapeutic option, especially considering that few specific types of epigenetic

drugs have shown to improve different neuronal phenotypes, as well as the early neuropathy associated with neurodegenerative diseases (Kwon et al., 2017).

Conclusion

Historically, breakthroughs in genetics and genomes have revolutionized our knowledge of human disease; now, epigenetics and epigenomics are ushering in the next stage of this revolution (Qureshi & Mehler, 2011). The pathophysiological mechanisms of complicated diseases have been drastically altered as a result of recent findings in epigenetics. There is mounting evidence from a wide range of disease models, from cell lines to postmortem human brains, showing epigenetic dysregulation plays a significant role in neurodegenerative illnesses. Reversing epigenetic modifications may have therapeutic benefits, and their role in brain illnesses is still being studied (Bertogliat et al., 2020). Many tiny chemical modulators, including activators and inhibitors, have been discovered to target the epigenetic enzymes in this dysregulation. In the future years, these compounds, either singly or in combination, may pave the way for a new line of research in neurotherapeutics. Furthermore, the chemical structures of many already used neurotherapeutics can be analyzed for potential use in the development of novel medications that target epigenetic enzymes (Singh et al., 2018). Finally, epigenetics may be the missing link in unraveling the complicated etiology of neurodegenerative diseases, and if the mechanism of these changes continues to be revealed, it may be possible to intervene therapeutically by reversing the epigenetic dysregulation associated with these conditions (Marques et al., 2011).

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