Narrative Review Article

Epigenetic Biomarkers In Early Alzheimer's Diagnosis In Aging Populations

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ABSTRACT

Epigenetics reveals how gene expression can be modified through the interaction between environmental influences and genetics, without altering the underlying DNA sequence. The main epigenetic mechanisms include DNA methylation, histone modifications, miRNA gene silencing, and mitochondrial epigenetics. These mechanisms can activate or repress genes, changing cellular processes, which increase the risk of errors in DNA, leading to mutations that can also be carried through generations. These epigenetic modifications have been linked to many diseases, like mental health disorders, diabetes, cancer, and Alzheimer's Disease (AD). The most common form of dementia is AD, and it is known for its cognitive decline, memory loss, and behavioral decline. AD accounts for 60-80% of the dementia cases and is predicted to affect 13.8 million individuals globally by 2060. At a molecular level, it involves the extracellular accumulation of β-amyloid (Aβ) plaques, leading to neuroinflammation, neuronal loss, and synaptic dysfunction. Current methods of diagnosis include neuroimaging and cerebrospinal fluid (CSF) biomarkers, which detect the disease after substantial neurodegeneration has occurred in late-stage AD patients. Epigenetic changes present a new avenue for early detection of AD using patient samples such as brain tissue, CSF, or blood. This paper examines DNA methylation and histone modifications in AD-related genes, such as *PSEN1* (Presenilin 1) and *APP* (Amyloid Precursor Protein), which influence inflammation, neuron survival, and tau phosphorylation. Studying epigenetic changes provides hopeful opportunities for early diagnostic and personalized treatment strategies.

Keywords: Epigenetics; Biomarker; Alzheimer's; Detection; Methylation; Histone; Aging; Neurodegeneration

INTRODUCTION

Epigenetics studies how genes can be turned on or off without modifying the underlying DNA sequence. It understands how individuals can be impacted by environmental influences and lifestyle choices that may turn their genes on or off. This affects how the body interprets and responds to its genetic code. Understanding the causes and depth of Epigenetics is a rapidly growing field of research, as it explains how genes and the environment interact to shape development, disease, and its risk. Epigenetic changes have specifically been linked to various conditions and diseases such as diabetes, cancer, Alzheimer's Disease, and mental health disorders. Because these

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changes can be reversible, Epigenetics offers a range of potential avenues for new treatments. Additionally, our lifestyle choices may impact not only our health but also our future generations. Evidence is accumulating to indicate that some epigenetic changes can be inherited by offspring, including epigenetic inheritance that may contribute to cognitive decline and AD in offspring (1). Epigenetics plays a crucial role in advancing personalized medicine by tailoring treatments to each individual's genetic expression, determining whether their genes are turned on or off.

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disorder characterized by memory loss, cognitive impairment, and behavioral disturbances. It is the most dominant form of dementia in aging populations, patients over 65 years, or early patients under 65 years (2). In all dementia cases, 60-80% is accounted for by AD, with a prediction of affecting 13.8 million people globally by 2060 (3-5). The pathogenesis of AD is multifaceted, including various environmental influences and genetic tendencies. In terms of molecular pathology, the extracellular accumulation of β-amyloid (Aβ) peptides and the intracellular cluster of the hyperphosphorylated tau protein result in neurofibrillary tangles (NFTs), abnormal thread-like structures found within the neurons (6). These pathological properties can lead to synaptic dysfunction, neuroinflammation, and widespread neuronal loss in the hippocampus and cerebral cortex. Clinical diagnosis of AD is based on overarching neuropsychological assessments, and both functional and structural neuroimaging (e.g., positron emission tomography (PET) and magnetic resonance imaging (MRI)), and biomarker analysis through the CSF which usually shows a decrease in Aβ42 levels (a peptide fragment that is a key biomarker in the diagnosis of AD) and increased total tau (t-tau) and phosphorylated tau (p-tau) (3). However, these levels only detect the disease after substantial neurodegeneration has occurred. There is an increasing interest in the development of minimally invasive biomarkers that may provide earlier diagnosis and more accurate detection of AD through its presymptomatic and symptomatic stages.

There are four main epigenetic changes linked with AD. The most common of these are DNA methylation and histone modifications. DNA Methylation is when a small chemical tag called a methyl group is added to the DNA. This can turn the genes on or off, affecting how they are used without changing the underlying DNA code. Histone modification occurs when a histone (a type of protein in chromosomes that DNA wraps around) undergoes epigenetic changes, including methylation, acetylation, and phosphorylation. The other two epigenetic changes linked with AD are microRNA (miRNA) gene silencing and mitochondrial epigenetics. Epigenetic alterations can pave the way for the onset of clinical symptoms, so they are increasingly recognized as potential early biomarkers for AD. These modifications, shaped by environmental exposures and genetic tendency, offer a unique opportunity to detect symptoms before neurodegeneration can occur.

This literature review describes the epigenetic changes specific to AD and their relevance in the early stage of Alzheimer's, and their adaptability across patient populations and tissue types. Overall, this paper highlights the important role of epigenetic biomarkers in advancing early detection of neurological disorders and the clinical outcomes of AD. Researchers are increasingly focusing on identifying early-stage biomarkers that appear before the onset of advanced symptoms. Epigenetic changes are important for this role because they can identify early molecular disruptions in brain function and are influenced by both a person's environment and their genes. The changes may occur years before clinical symptoms, making them valuable for early detection. The objective of this paper is to explore epigenetic modifications that contribute to AD and the potential for their use as biomarkers for diagnosis.

EPIGENETIC MECHANISMS AS EMERGING BIOMARKERS IN ALZHEIMER'S DISEASE

This section explores the principal epigenetic mechanisms associated with Alzheimer's disease in depth, beginning with the four primary types of modifications—DNA methylation, histone modifications, miRNA gene silencing, and mitochondrial epigenetics (Figure 1)— primarily focusing on the first two. It then looks at how these changes affect the expression of specific AD-related genes, such as APP and PSEN1. This section describes the molecular pathways interfered with by these epigenetic alterations, such as inflammation, neurogenesis, and tau phosphorylation. Additionally, it examines the detectability of these epigenetic changes in various patient samples, such as brain tissue, blood, and CSF. Finally, it highlights their unique advantages and inherent limitations.

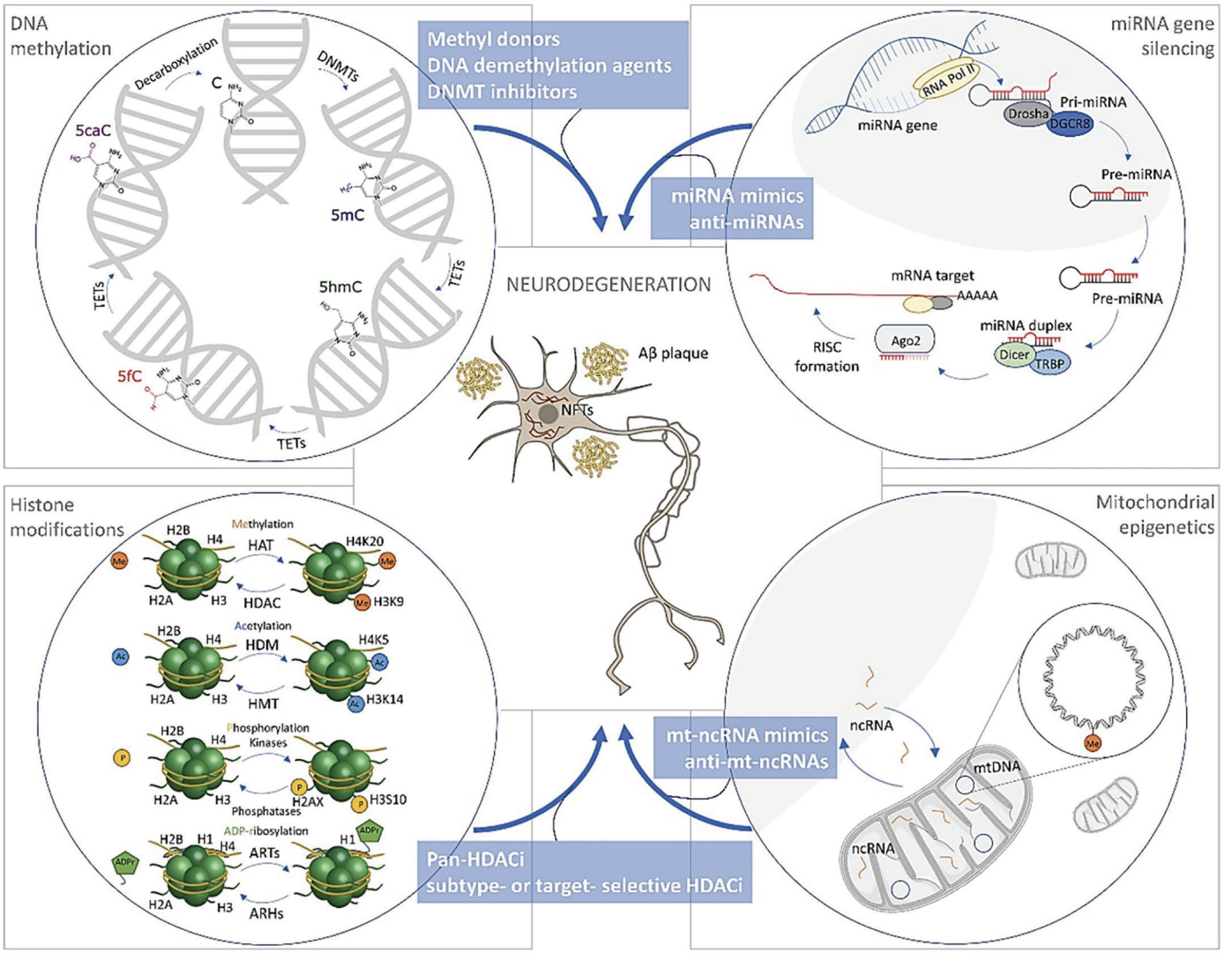

Figure 1. This image is sourced from the paper "Epigenetics of Alzheimer's Disease" (7). It demonstrates the four epigenetic modifications linked to neurodegeneration: DNA methylation, miRNA gene silencing, histone modification and mitochondrial epigenetics.

Types of Epigenetic Modifications Linked to AD

DNA methylation

The most widely studied and well-understood type of epigenetic regulation is DNA methylation (Figure 1 upper left). This modification can affect gene expression by altering the transcription process, which converts a gene's genetic information into RNA. It involves adding a methyl group to the 5th carbon of a cytosine base in the DNA (7, 8). This reaction is performed by DNA methyltransferases (DNMTs), an enzyme, using a molecule named S-adenosylmethionine (SAM) as a methyl donor (9-11). Most methylation in humans happens on cytosines within CpG islands, located in the promoter regions of the genes. Our human genome has about 45,000 CpG islands and 28 million CpG sites (12). Methylation in gene bodies can change over time through the action of TET enzymes. Regular methylation using 5mC occurs in gene bodies, but when it converts to 5hmC (5-hydroxymethylcytosine), another layer of protection is added, allowing 5hmC to control the extent of gene expression (7, 13).

CpG islands are usually unmethylated and found near gene promoters so transcription factors can bind (14). After these islands become methylated, they slightly change the DNA structure and block transcription factor binding, obstructing gene expression (15). Once the DNA is methylated, it can attract MBDs or methyl-CpG binding proteins, which add other molecules and cease gene activity (16).

DNA methylation is important during early development as it helps cells respond to the environment eventually in life (16).

Histone Modifications

The second most widely studied epigenetic regulation is histone modifications (Figure 1 bottom left). This involves histone proteins (H1, H2A, H2B, H3, and H4) that form the fundamental chromatin when DNA wraps around histones (17, 18). These modifications occur on the histones' N-terminal "tails" and are crucial for regulating how tightly the DNA is wrapped around them. Through this process, the genes that are turned on or off are decided (17, 19). The most common modifications include acetylation, phosphorylation, methylation, and ubiquitination (9, 17, 18).

For example, enzymes like histone acetyltransferases (HATs) add acetyl groups, which loosen the chromatin structure, making DNA expression easier. On the contrary, histone deacetylases (HDACs) remove these groups, causing the DNA to become tightly packed, blocking or "silencing" gene expression (16, 17). Histone methylation is controlled by methyltransferases and demethylases, which can activate or repress genes depending on the location and the amount of methyl groups added (7, 16). These chemical changes do not change the DNA sequence, but they affect how the genes are expressed by the cell. This concept is known as the "histone code," in which various combinations of histone modifications control gene expression in multiple ways (7). In AD, abnormal histone modifications in some cases may be linked to the breakdown of chromatin structure, leading to improper gene expression, particularly in brain cells affected by tau protein accumulation (21). This is frequently triggered by oxidative stress and DNA damage, contributing to neurodegeneration in Alzheimer's patients (21, 22).

miRNA Gene Silencing

miRNAs or microRNAs are short, single-stranded non-coding RNA molecules (Figure 1 upper right). Therefore, they are RNA sequences that do not code for proteins, but they function as regulators for gene expression after transcription (7). miRNAs are usually 19-24 nucleotides long and "silence" specific mRNAs by advancing their degradation or by blocking their translation to proteins (22, 23).

This process begins in the nucleus, where miRNAs are transcribed as RNA molecules known as primary miRNAs or pri-miRNAs (7). Pri-miRNAs have a long structure similar to a hairpin. An enzyme, Drosha, and a protein cofactor known as DGCR8 cut the pri-miRNA into a smaller molecule called precursor miRNA (pre-miRNA) (23). Then, the pre-miRNA is transported to the cytoplasm (the fluid part of the cell surrounding the nucleus), where a different enzyme, Dicer, processes it into smaller double-stranded RNA molecules (24). A strand called the guide strand is loaded into a protein complex called RISC (RNA-induced silencing complex). The other strand, known as the passenger strand, is typically degraded (25). After the miRNA is incorporated into RISC, it guides the complex to the target mRNA and binds to a complementary sequence in the 3' untranslated region of mRNA.

This process allows cells to tune gene expression effectively. Because miRNA can regulate hundreds of mRNAs, its effects are mediated through epigenetic mechanisms.

Specifically in AD, miRNA gene silencing disrupts normal gene regulation. Some miRNAs are reduced, but others that promote neurodegeneration are increased. Due to this imbalance, synaptic function, tau regulation, and amyloid-beta clearance increase, leading to symptoms such as memory loss. Even slight shifts in miRNA levels affect multiple genes simultaneously, making their role in AD widespread and significant.

Mitochondrial Epigenetics

Mitochondrial epigenetics is crucial in the development of AD, as it disrupts the cell's ability to generate energy (Figure 1 bottom right). Mitochondria are essential for cellular energy production through the process of oxidative phosphorylation. This pathway becomes damaged in AD, leading to an increase in the production of reactive oxygen species and the activation of cell death pathways, known as apoptosis (26). Mitochondrial DNA (mtDNA) is unique because it lacks introns and nucleosomes, relying on nucleoid structures for packaging (7). Mitochondrial DNA is also only passed down from the mother, with its own set of genes still depending on nuclear-encoded proteins

to function. In AD, mutations in mtDNA, deletions of mtDNA, and changes in the D-loop have all been observed (27, 28). These alterations interfere with the expression and replication of mtDNA, reducing mitochondrial efficiency and increasing cellular stress. As epigenetic-like mechanisms influence mtDNA regulation, these disruptions are studied as a form of mitochondrial epigenetic dysregulation in AD (7).

Specific Genes Affected by Epigenetic Changes in AD

APP (Amyloid Precursor Protein)

The amyloid precursor protein (APP) plays a vital role in Alzheimer's disease (AD), particularly in early-onset forms. APP is a protein found in the brain at the synapse region, where it reinforces the neural plasticity, ensuring neurons communicate and connect. Under standard circumstances, APP is broken down by enzymes. When it is processed by alpha-secretase (an enzyme that processes APP), the byproducts are safe (29). Although gamma-secretase and beta-secretase cleave and split APP, they produce a toxic segment called amyloid-beta (Aβ), specifically the Aβ42 variant (30). Aβ42 tends to aggregate into plaques or sticky clumps, toxic to neurons, and a key feature of AD (30).

The APP gene is located on chromosome 21. It is common in individuals diagnosed with Down syndrome because they usually develop Alzheimer's-like symptoms by their 40s, caused by the overexpression of APP (30). These mutations in the APP gene can lead to familial early-ons*et al*zheimer's, with currently over 69 identified missense mutations. A missense mutation is a type of mutation where one nucleotide change results in a different amino acid being incorporated into the original protein. This mutation occurs near the gamma-secretase site, resulting in increased production of Aβ42. For instance, the London mutation (V7171) and the Swedish mutation increase levels of Aβ42, thereby enhancing the formation of plaque. Another mutation, L723P, found in an Australian family, increases Aβ42 levels (30). These experiments show how changes in APP processing contribute to the development of AD.

Studies have shown that epigenetic modifications in DNA methylation regulate the APP gene and can contribute to the early development of disease. Usually, the APP gene is methylated in its promoter region, which keeps its expression low. During AD, this promoter region becomes hypomethylated, causing the gene to become more active. This leads to faster and more APP protein production, causing higher levels of amyloid-beta accumulation in the brain (31). APP hypomethylation is an early epigenetic marker of AD, as this shift in methylation occurs before clinical symptoms appear. Potentially, these changes can be detected in tissues such as cerebrospinal fluid and blood, thereby increasing the value of using APP methylation as an early-stage biomarker for diagnosing AD (31).

APP's role in the pathogenesis of AD is well established; further research is needed to clarify the methylation status of its gene, given the numerous conflicting studies. Some studies have found that the APP promoter is hypomethylated in the neurons and glia of AD patients, while others have reported no change (32). A common obstacle faced when harvesting and examining cortex tissue is that the cell-type composition changes upon aging or disease progression. In another study focusing on late-ons*et al*zheimer's disease (LOAD), they address this obstacle by grouping cells into neuronal and glial cortices from 31 healthy donors with Braak stages established. Through this comparison of bulk tissue and cell classification, the level of detection of disease-related changes increases (33). This also reveals that neurons and glial cells undergo significant epigenetic alterations during AD. In particular, glial genes show strong age-related epigenetic signs that neuronal genes, hence highlighting the critical role of neuron-glial interactions in shaping AD pathology (34).

However, this data does not remain consistent across all studies. Some experiments and researchers have reported no significant differences in APP gene methylation in control brains and AD (31). This inconsistency can be caused from variations in the type of tissue analyzed (whether it is bulk brain tissue or compared to solely glial or neuronal cells), variability in disease stage among patients (for example late-onset versus early-onset AD) (31-33). The presence of APP hypomethylation in many studies shows a potential role in the early pathogenesis of AD.

In summary, the epigenetic regulation of the APP gene through DNA methylation may play a crucial role in Alzheimer's disease, especially during the early stages before symptoms become more prevalent. While genetic mutations in APP are well-established in familial AD, epigenetic mechanisms like hypomethylation may also drive abnormal APP expression and amyloid-beta accumulation in irregular cases. Despite some conflicting findings, the growing body of evidence indicates that tracking changes in APP promoter

methylation could become a valuable strategy for early diagnosis. As epigenetic research expands and detection methods become more sensitive, APP methylation status may help pave the way for earlier and more accurate detection of Alzheimer's disease (30, 31).

PSEN1 (Presenilin 1)

Presenilin 1 (PSEN1) is a gene located on chromosome 14, which encodes a polytopic membrane protein that spans the lipid bilayer multiple times. In addition to being a structural protein, it plays a crucial role in an enzyme complex known as γ-secretase. γ-secretase, also known as gamma-secretase, is a crucial protein complex that cleaves specific membrane proteins, most notably the APP and another protein called Notch, both of which are key to cellular communication (30). In this complex, PSEN1 is a catalytic component, meaning it helps "cut" γ-secretase. γ-secretase cannot function correctly without PSEN1 disrupting cellular pathways.

Mutations in PSEN1 are a prime cause of early-onset familial Alzheimer's disease (EOFAD). In familial Alzheimer's disease, an individual has a higher chance of developing the condition because of inherited genetic mutations before the age of 65. A majority of these mutations are missense mutations, meaning one or more amino acids in the protein are replaced. The consequences of a missense mutation can be extensive. As a result, the γ-secretase complex starts producing a more extended, stickier version of amyloid-beta called Aβ42, instead of the typical and less harmful Aβ40 (30, 31). Because Aβ42 is highly aggregation-prone, it clumps and forms amyloid plaques in the brain, a signifying synonym of Alzheimer's disease. This imbalance in the Aβ42/Aβ40 ratio makes PSEN1 a highly valuable biomarker for detecting and understanding AD disease in its early stages (35).

Overall, 300 different PSEN1 mutations have been identified, and a majority of them increase the amount of Aβ42 produced (30, 35). M146L, a well-known mutation, was discovered in a family from southern Italy. This mutation causes symptoms of Alzheimer's to begin from 43 years of age, which is significantly earlier than the typical onset of AD in general populations (35). Carriers of the M146L mutation exhibit an apparent autosomal dominant inheritance pattern, indicating that only one copy of the mutated gene is required to develop the disease. Furthermore, harmful changes caused by the PSEN1 mutation can be detected outside the brain. For example, fibroblast cells derived from a patient's neurons or skin still exhibit elevated Aβ42/40 ratios (35). This opens up possibilities for early diagnosis and testing in living individuals long before symptoms begin.

Scientists have experimented using animal models and lab-grown cells to uncover the mechanisms behind PSEN1 mutations. In mouse models, researchers have modified or deleted the PSEN1 gene to observe how memory and learning are affected, as well as the basic symptoms of AD. As a result, the mice exhibited mild cognitive impairments in tasks that required long-term memory and spatial navigation (30). Conditional knockout studies (where PSEN1 is turned off in specific brain regions) have shown that the gene plays a crucial role in memory shaping and retention. In a different experiment, induced pluripotent stem cells (iPSCs) were derived from patients with the PSEN1 mutations to create neurons in a dish. These neurons offered a human-based model, showing the same elevated Aβ42 levels and plaque-like aggregates (35).

Numerous real-world case studies illustrate the far-reaching impact of PSEN1 mutations. The N135S mutation, from a Greek family, displayed unusual symptoms, including memory loss, limb stiffness, and seizures. This suggests that PSEN1 mutations can affect both cognition and motor control (30). Another PSEN1 mutation studied in an African family was found to cause rapid disease progression. Additionally, during autopsy, the individual showed signs of neuronal loss, neurofibrillary tangles, amyloid plaques, and even brainstem degeneration (30). These studies and observations demonstrate that PSEN1 mutations increase Aβ42 production, while also influencing disease duration, severity, and various other symptom presentations.

One of the most significant biological consequences of PSEN1 mutations is the alteration in the shape and function of the γ-secretase complex. Research has shown that some PSEN1 mutations lead to a "tighter" structure of the presenilin-1 protein (PSEN1). This causes the two ends of the protein, the N-terminus and C-terminus, to be pulled closer together, affecting how APP is recognized and eventually skewing production of Aβ40 towards Aβ42 (35). Nevertheless, not all PSEN1 mutations function in the same manner. Some mutations result in partial loss of a function, where the protein still works but not as efficiently as usual.

In contrast, others redirect the protein's ability to recognize substances like APP (35). Studies also mention that some limitations of human studies are that

they only assess CpG methylation, but methylation also takes place at cytosine moieties (36). These experiments and insights are crucial to understanding EOAD and developing targeted drugs that combat these harmful changes.

Molecular Pathways and Mechanistic Functions

Tau Phosphorylation and Aggregation

In Alzheimer's Disease (AD), tau phosphorylation and aggregation are key pathological processes. Tau is a protein associated with microtubules, and it stabilizes the internal staging of neurons. In AD, tau undergoes hyperphosphorylation, the unrestricted addition of phosphate groups, disrupting its ability to bind to microtubules (7). This causes tau to misfold and aggregate into toxic neurofibrillary tangles. During tau aggregation, the misfolded tau molecules clump and interfere with neuronal transport, eventually leading to cell death. Histone deacetylases (HDACs) are epigenetic regulatory enzymes that remove acetyl groups from histone and non-histone proteins, influencing the processes (7). HDAC6 is known as a class II HDAC, affecting tubulin acetylation, inflammatory pathways, tau degradation, and tau phosphorylation (37, 38). On the other hand, reducing HDAC6 levels promotes tau clearance while decreasing aggregation, helping neuronal survival (39, 40). However, overexpression of HDAC6 reduces α-tubulin acetylation, a chemical modification that increases microtubule stability, causing impaired mitochondrial transport and destabilized microtubules (7). HDAC4 is similar to HDAC6, as it reduces damaged memory. When overexpressed, HDAC4 triggers apoptosis, and when inactivated, it prevents neuronal death (41). Class III HDACs, better known as sirtuins, aid in learning and memory, specifically in AD (38). SIRT1, in AD, is reduced in the parietal cortex, correlating with tau accumulation and amyloid-β, driving tau aggregation (39).

Amyloid Precursor Protein (APP) Processing/Aβ Production

The APP and Aβ production play a key role in Alzheimer's disease. MiR-9, a type of microRNA, helps with movement, growth, and the specialization of neural progenitor cells (7). When downregulated, miR-9 can suppress BACE (beta-site APP-cleaving enzyme), a protein that helps to produce Aβ, causing increased Aβ accumulation (42). Conversely, downregulating miR-9 affects CAMKK2, activating the CAMKK2-AMPK pathway, leading to higher levels of p-tau (phosphorylated tau) and excess amyloid (7). Through various findings, it is known that amplifying miR-9 can influence genes like TRIM2, SIRT1, and TGFB1, depending on the disease stage (7). Histone modifications also impact how APP is processed. HDAC inhibitors are drugs that prevent the removal of acetyl groups from histones (7). They have been shown to decrease Aβ production, improve memory, and lower the phosphorylation of tau. These examples clearly show how epigenetic changes can affect molecular pathways that navigate AD.

Synaptic Plasticity and Memory Formation

Synaptic plasticity is the brain's ability to connect between neurons in response to activity, either strengthening or weakening. It also aids with memory formation, the process of encoding, recovering, and storing information. DNA methylation maintains cellular functions and regulates synaptic plasticity, affecting cognitive capability (43). Correspondingly, DNA hydroxymethylation is a modification of methylated DNA and is crucial for neurodevelopment and memory-related processes (44). HDACs impact these processes as well. The reduction of HDAC4 damages memory and learning, but overexpression leads to apoptosis (41). Sirtuins are also regulators of memory and synaptic plasticity and have been linked to AD (39). miRNAs regulate synaptic function. BDNF is a protein vital for synaptic plasticity that induces miR-132, which targets MeCP2 to increase BDNF levels and facilitate memory formation (7, 45). Various other miRNAs like miR-9, miR-137, miR-181c, and miR-129 control genes involved in synaptic plasticity (46, 47). HDACi (HDAC inhibitors) reduce amyloid-beta accumulation and tau phosphorylation, thereby enhancing memory and synaptic plasticity (9, 48). These modifications at the DNA and histone levels contribute to the complex processes of learning and memory.

Inflammatory Signaling

Histone and DNA changes are epigenetic modifications crucial to the regulators of inflammatory signaling in neuronal differentiation and cognitive functions in many CNS diseases (10, 49, 50). In Alzheimer's, epigenetic alterations in brain regions can trigger abnormal inflammatory responses, causing overactivation of microglia and amplification of CNS inflammation, contributing to the pathological continuation of AD (49, 51). Neuroinflammation,

a defense mechanism, becomes harmful when sustained but only promotes neurodegeneration, worsening amyloid-β (Aβ) and tau pathology (4, 17). Understanding the relation of epigenetic regulation and inflammatory responses to AD provides insight into the disease's foundational cracks.

Epigenetic biomarkers vs Traditional biomarkers

There are various advantages that epigenetic markers provide for diagnosing and comprehending Alzheimer's disease. Epigenetic changes are reversible, unlike genetic mutations. Therefore, they create future opportunities for treatment and prevention strategies (7). These epigenetic changes occur before clinical symptoms show up, making them precious and an important field of study for early detection tools (32). Tissues like blood and saliva can be tested to detect these changes, allowing for non-invasive testing methods instead of relying on post-mortem diagnosis, CSF, or brain tissue (9). Analyzing epigenetic patterns shows the interaction between genes and influences in the environment while providing an overview of the disease's progression (10). This advantage allows for personalized medicine for each patient's epigenetic history (14). Overall, understanding and investing further research in epigenetic biomarkers provides a better understanding of the early onset of diseases, learning the process of the disease, and diagnosing it.

POTENTIAL OF EPIGENETIC BIOMARKERS FOR EARLY DETECTION ACROSS ALZHEIMER'S STAGES

This section evaluates how effective the biomarkers discussed in subtopic 1 are in diagnosing different AD at early stages of disease progression. It also considers other forms of AD where memory loss is not the first symptom. This section will discuss how early markers appear and how they can provide guidance in the patient's journey.

Stage-Specific Expression of Epigenetic Markers

Stage-specific expression of epigenetic biomarkers in AD focuses on how molecular changes differ from the stages in AD, specifically in EOAD, where symptoms may not be as well-known or not always associated with memory decline. For example, one study analyzed tissue from the hippocampus, an area known to be vulnerable to AD. They identified 118 Differentially Methylated Positions (DMPs) with early signs of pathology (52). These shifts in methylation in genes are linked to chromatin regulation and fatty acid metabolism, which change, leading to cognitive decline (52). Conversely, peripheral studies examined mitochondrial DNA (mtDNA), specifically D-loop methylation and gene-specific markers such as APOE and Brain-Derived Neurotrophic Factor (BDNF), which revealed variations in patients with mild cognitive impairment (MCI). Although findings are inconsistent for BDNF, some groups observed an increase in methylation, while other studies did not (53). Another review strongly suggests that both DNA methylation, histone modifications, and non-coding RNAs contribute to expression patterns that are stage-dependent, with changes detectable in fluids like CSF and blood (54). Hippocampal tissue studies provide strong evidence for consistent and early methylation changes (52), whereas peripheral markers lack replication and consistency across studies (53). This highlights the need for large-scale studies to determine whether peripheral markers can accurately reflect changes in the brain for mild AD stages and EAOD, particularly in atypical forms where memory loss is not the initial symptom (54). Specific examples of atypical forms are posterior cortical atrophy (PCA) with visuospatial degeneration, and logopenic variant of primary progressive aphasia (lvPPA) detected through language deficiencies. These forms involve clearly distinct epigenetic patterns like specific region DNA methylation linked to neuronal vulnerability (3). In PCA, methylation changes are found in the parietal and occipital regions of the brain, which are responsible for visual processing, leading to visuospatial defects. On the other hand, IvPPA shows methylation changes in the left temporoparietal regions, which are responsible for language processing and memory, thereby affecting character speech and language memory.

Biomarker Sensitivity and Specificity in Early Detection

Studies on EOAD epigenetic biomarkers indicate that molecular changes can be detected before noticeable cognitive decline occurs. DNA methylation in the promoter regions of COASY and SPINT1 has been studied to show an increase in patients with amnestic mild cognitive impairment (aMCI) and AD. COASY demonstrates a high sensitivity of 96.6% with a specificity of 96.75% for identifying prodromal stages (55). Traditional biomarkers like the CSF, tau proteins, and neuroimaging techniques have high sensitivity and specificity but are less feasible for repeated early

screening because they are invasive (56). Blood-based miRNAs are a stable epigenetic biomarker in circulation and can recognize early neuronal changes in AD. Analyses indicate a sensitivity of 0.89 and specificity of 0.85 for miRNA biomarkers in MCI detection, exhibiting strong potential for early-stage diagnosis (57, 58). Tissue-based methylation, such as that of COASY, demonstrates strong potential for early detection. At the same time, peripheral biomarkers, like blood miRNAs, offer accessibility but have slightly lower consistency across studies, resulting in a gap in reproducibility and validation (58). Together, these findings demonstrate that examining both peripheral and central epigenetic markers can enhance early diagnostic accuracy. Still, large-scale studies are needed to determine their reliability for early AD stages.

Role of Epigenetics in Predictive vs. Diagnostic Use

Many system-level analyses, including epigenomic, transcriptomic, genomic, and metabolomic data, suggest that multomic panels can provide reliable and informative predicted biomarkers (59). For example, the identification of mutations in genes such as APP and PSEN1 for early-onset AD demonstrates the high complexity of epigenetic and genetic contributions to disease onset (59). This means that while some mutations can trigger EOAD, epigenetic modifications can further change how the same genes are expressed. While these findings highlight the promise of early detection, current clinical tools are not sensitive enough to detect slight subtle changes in preclinical stages of the disease, creating a gap in predictive biomarkers that could guide early diagnoses (59). The collective failures of drugs in late-stage trials underscore the need for biomarker selection of patients in preclinical and early AD stages (59).

Advancements in various sequencing technologies support the possibility of prophylactic interventions before symptom detection through the identification of predictive biomarkers (60). Pharmaccogenetics and pharmacoepigenomics tailor drug therapies to individual patients by examining epigenetic variability, which influences drug response (60). These approaches are promising, but gaps remain in taking biomarker discovery into complete clinical practice application with longitudinal validation (60). This can help understand how well epigenetic markers reflect changes in the brain of an AD patient and other neurodegenerative diseases. These experiments highlight that studying early epigenetic biomarkers and personalized medicine can enhance the prediction and advancement of technology for the diagnosis of AD. Still, it is crucial to address and overcome the current limitations.

Integration with Imaging and Cognitive Testing

The ADNI-1 study focused on longitudinal MRI and PET imaging. They examined the structural and functional brain changes over time, specifically the atrophy of the hippocampus, to detect progression in MCI and early AD (61). When used with early epigenetic biomarkers, the imaging measures can provide complementary power with further study. Before structural brain changes are noticeable, epigenetic biomarkers can also detect molecular dysregulation, facilitating early imaging and testing (61). This approach could significantly enhance the effectiveness of early-stage detection. However, it is crucial to bridge the gap between large-scale imaging studies and incorporate longitudinal measurements to generalize the predictive accuracy across cohorts (61).

Evidence from FAD studies supports this potential. A longitudinal study of PSEN1 mutation carriers, CSF sampling, MRI, and PET imaging was used to capture disease progression over generations (62). Epigenetic biomarkers from the blood or CSF could support the sensitivity of these measurements by identifying possible mutations and early molecular changes before the disease escalates. Challenges remain due to the limited sample size, and repetitive MRI scans could cause claustrophobia in patients (62).

RELIABILITY AND GENERALIZABILITY OF EPIGENETIC BIOMARKERS IN DIVERSE POPULATIONS

This section assesses the consistency of epigenetic biomarkers across different populations, including aging versus early patients, women versus men, and various ethnic groups. It also explores reliability in brain tissue, blood, and other limitations.

Sample Source and Biomarker Reliability

DNA methylation (DNAm) plays distinct roles in the brain, varying by sex and age (63). A meta-analysis conducted on publicly available DNAm datasets from four different brain regions—temporal, frontal, entorhinal cortex, and cerebellum—shows significant differences between males and females (63). Aging affects the cortical regions differently

from the cerebellum. AD-related epigenetic changes typically occur at the same sites with age, and whether methylation increases or decreases is closely related in both AD and aging. This suggests that epigenetic changes in AD can speed up the process (63). Overall, this shows that both age and sex influence brain epigenetic patterns, but age plays the bigger role in the changes seen in AD (63).

Sample Source and Biomarker Reliability

The reliability of epigenetic biomarkers is thoroughly dependent on the type of biological sample chosen. Studies have shown that the most accurate source remains brain tissue because it clearly reflects the molecular alterations in the CNS (53). Postmortem brain studies have identified reproducible changes in DNA methylation and other epigenetic marks associated with AD pathology, making brain tissue a key standard for mechanistic comprehension (53). However, the use of postmortem brain tissue restricts its application for longitudinal living patients or early diagnosis (53).

The cerebrospinal fluid (CSF) is in close contact with the brain, therefore providing another source of study. Studies using CSF can capture signs of neurodegeneration more distinctly than peripheral samples such as blood(53). Some evidence suggests that a few blood methylation changes in BIN1 and APOE are correlated with CSF levels of tau and amyloid-β (53). Therefore, CSF is another valuable source as it bridges the gap between central pathology and peripheral findings (53). CSF samples require a process called a lumbar puncture, where a needle is inserted into the lower back to collect spinal fluid (53). Because collecting CSF samples currently remains risky, it makes regular check-ups and large-scale studies more challenging.

Peripheral blood remains the most widely studied sample and most accessible for epigenetic biomarker research. Through peripheral blood, various alterations in DNA methylation in AD-affected brain cells may be mirrored in blood samples(53). Blood biomarkers are particularly valuable because they can be collected multiple times, making them effective for longitudinal studies and repetitive check-ups (53). Additionally, peripheral epigenetic profiles can be influenced by various characteristics, including aging, environmental factors, and lifestyle (53). When compared to central markers, peripheral blood holds a strong potential as a reliable tool for early detection and tracking the progression of AD.

CONCLUSION

Epigenetic mechanisms, including histone modifications, DNA methylation, miRNA regulation, and mitochondrial changes, are crucial in the pathogenesis of Alzheimer's disease. By regulating the expression of genes such as APP and PSEN1, these processes ultimately lead to the accumulation of tau pathology, amyloid-beta, and neuroinflammation, which contribute to disease progression. They may be able to detect abnormalities at a molecular level before clinical manifestation. When studied through cognitive testing and neuroimaging, epigenetic markers hold promise for future specificity in the early detection of AD, while supporting possible personalized therapeutic strategies.

The use of multiple sample sources, ranging from post-mortem brain tissue, blood, and CSF, can increase the applicability of epigenetic biomarkers for AD. Brain tissue provides insight into central pathology, but CSF and blood samples remain safer and more effective methods for monitoring disease progression in living patients. By combining both central and peripheral biomarkers, researchers can enhance both patient safety and specificity in detecting Alzheimer-related changes. Using this multi-sourced approach enables longitudinal studies and facilitates better tracking of disease progression.

Nevertheless, challenges remain before these findings can be applied to medical settings and clinical practice for patients. Due to the diversity among tissue sources, disease stages, and patient populations, establishing a universal, reliable biomarker is challenging. Therefore, large-scale studies are necessary for the validity and reproducibility of these findings. Additionally, the overlap between epigenetic changes associated with normal aging and those specific to AD needs to be refined to ensure accurate diagnostics. Despite these obstacles, the study of epigenetics is a thriving field of study and offers a powerful new approach to detecting AD earlier and more accurately, allowing for earlier effective treatment and better outcomes for aging individuals who are at risk. Current advancements in pharmacoepigenomics and biomarker integration emphasize the clinical management of AD. The field of epigenetics in AD is a captivating pathway offering effective patient outcomes with future research.

CONFLICT OF INTERESTS

The author declares that there are no conflicts of interest related to publication of this work.

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