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DNA Methylation in Aging and Alzheimer's Disease

Raymond Cheng[a], **Jingmin Shu**[b], **Hai Chen**[c], **Ming Li**[d], **Xiaodong Cheng**[e], **Li Liu**[f]

[a] Case Western Reserve University, Cleveland, OH 44106, USA.

[b] College of Health Solutions, Arizona State University, Phoenix, AZ 85004, USA. Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, AZ 85281, USA.

[c] College of Health Solutions, Arizona State University, Phoenix, AZ 85004, USA. Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, AZ 85281, USA.

[d] University of Arizona College of Medicine, Phoenix, AZ 85004, USA.

[e] Texas Tech University Health Sciences Center, Midland, TX 79705, USA.

[f] College of Health Solutions, Arizona State University, Phoenix, AZ 85004, USA. Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, AZ 85281, USA.

Abstract

DNA methylation undergoes significant changes with age. These alterations play a pivotal role in the development of age-related diseases, including Alzheimer's disease (AD). Recent advancements in DNA methylome profiling have revealed global hypomethylation patterns, particularly within repetitive elements, as well as gene-specific changes that are associated with neurodegeneration. Age-related alterations in DNA methylation have been implicated in the disruption of key cellular processes, such as inflammation and proteostasis, both central to AD pathology. However, several challenges persist in this field. One major issue is the inconsistency of findings across different brain regions and tissue types, which complicates result interpretation. Furthermore, the limited understanding of cell-type specificity raises concerns about the generalizability of findings from bulk tissue analyses. Key questions remain in DNA methylation research related to aging and AD, including elucidating the precise mechanisms driving methylation changes, exploring cell-type specificity, determining the functional consequences of these alterations, and investigating cross-tissue correlations. A deeper understanding of the spatiotemporal dynamics of methylation changes, the underlying mechanisms, and their therapeutic implications is essential to the development of novel prevention and treatment strategies. This review will delve into these findings and challenges, offering insights into future research directions.

Corresponding Author: xiaodche@ttuhsc.edu, liliu@asu.edu.
Raymond Cheng, Jingmin Shu, Contributed equally to this work as the first authors.

Keywords

DNA; Methylation; Aging; Alzheimer's Disease

INTRODUCTION

Aging is a complex biological process that affects all humans, leading to a progressive decline in physiological functions and an increased susceptibility to diseases, including neurodegenerative disorders. In the brain, aging is associated with structural changes, cognitive decline, and increased risk of pathological conditions. Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with aging, characterized by a gradual decline in cognitive function. It currently affects 35.6 million people worldwide and is becoming an increasingly urgent concern as the global population continues to age [1]. Pathologically, AD is characterized by atrophy of the cortex and medial temporal lobe, including the hippocampus [2, 3], extracellular deposition of amyloid plaques [4], and intracellular neurofibrillary tangles [4]. Signs of neuroinflammation and angiopathy are also common findings in AD [5, 6]. The etiology of AD is multifactorial, involving a complex interplay of genetic, epigenetic, and environmental factors. Among these, DNA methylation, a key epigenetic mechanism, has attracted significant attention [7, 8].

DNA methylation is an essential epigenetic modification that plays a pivotal role in regulating gene expression and maintaining genomic stability. DNA methylation involves converting a cytosine residue to 5-methylcytosine (5mc) by the addition of a methyl group to the 5th carbon of cytosine residues in CpG dinucleotides, which is catalyzed by DNA methyltransferases (DNMTs), primarily *DNMT1, DNMT3A,* and *DNMT3B. DNMT1* maintains existing methylation patterns during DNA replication, while *DNMT3A* and *DNMT3B* are responsible for de novo methylation [9].

The methylation status of CpG islands, often located in gene promoter regions, is a critical determinant of gene expression [10]. Hypermethylation typically leads to gene silencing by impeding the binding of transcription factors or recruiting proteins that compact chromatin, whereas hypomethylation can result in gene activation or genomic instability. DNA methylation is essential for various biological functions, including development, genomic imprinting, and X-chromosome inactivation [10, 11].

DNA methylation also plays an important role in the pathogenesis of human diseases, such as cancer, neurological disorders, autoimmune diseases, and cardiovascular diseases [11–13]. For example, in the brain, DNA methylation is crucial for regulating neuronal function, synaptic plasticity, and memory formation, all of which are affected by aging and AD [14, 15]. Targeting DNA methylation has emerged as a promising therapeutic strategy [14]. DNA methyltransferase inhibitors, such as azacitidine and decitabine, have been used to treat diseases such as myelodysplastic syndromes and acute myeloid leukemia by reactivating silenced tumor suppressor genes [16]. These inhibitors are also being explored for their potential to modulate DNA methylation in AD, restore normal methylation patterns and gene expression, potentially alleviating symptoms or slowing disease progression [17]. Furthermore, lifestyle and dietary interventions that influence methylation patterns are under

investigation for their potential to reduce AD risk or delay onset. This review will examine the role of DNA methylation in brain aging and AD, highlighting key findings from recent studies, exploring the underlying mechanisms, and discussing potential clinical implications.

GLOBAL DNA METHYLATION CHANGES IN AGING AND AD

DNA methylation can be classified as either global or gene-specific. Global methylation reflects the average percentage of methylation across the entire genome, while gene-specific DNA methylation refers to the methylation level within a specific gene. It has been observed that there was a significant decrease in the overall content of 5mC, particularly in non-CpG (CHG and CHH) contexts, as part of the aging process. Multiple studies have demonstrated that global hypomethylation is associated with age-related phenotypes and diseases, making it a potential biomarker for biological aging [18–22]. The most pronounced effects of global hypomethylation are seen in repetitive DNA elements, such as LINE-1 and Alu sequences, which constitute a significant portion of the genome. These elements are usually heavily methylated to prevent their transposition, as their activation can lead to genomic instability [23]. As global methylation levels decline with age, these elements may become hypomethylated, potentially reactivating transposable elements and contributing to age-related genomic instability [24]. Several mechanisms have been postulated to contribute to the age-related decline in global DNA methylation, including altered DNA methyltransferase activity, exposure to oxidative stress, and nutritional and environmental factors during the process of aging [25, 26].

As the brain ages, misfolded tau and amyloid proteins accumulate due to a decline in proteostasis, a key molecular marker of aging [27]. Although this buildup occurs in older adults with normal cognition or mild cognitive impairment, it tends to be more pronounced in individuals with AD. One study using immunohistochemical methods to examine brain regions affected by AD observed global hypomethylation in the entorhinal cortex, but not in the cerebellum, a region known to be spared in AD [28]. Another study of monozygotic twins found a significant decrease in DNA methylation in the anterior temporal cortex and the superior frontal gyrus of those who developed AD, compared to their healthy siblings [29]. However, several other studies have reported an association between AD and increased global DNA methylation levels in the middle frontal gyrus and frontal cortex [30–32]. Studies focusing on the hippocampus, one of the earliest regions affected by AD, have also produced inconclusive results, showing both increased and decreased global methylation in AD patients [15, 33] (Fig. 1).

Peripheral blood samples are more accessible and less invasive to obtain than brain tissues. In one study of peripheral blood mononuclear cells (PBMCs), besides a significant increase in global methylation in AD patients, higher methylation levels were also observed among *APOE* ε4 allele carriers, who are at an increased risk of developing AD, than non-carriers [34]. However, a longitudinal study that collected PBMCs from community cohorts over 11–16 years revealed substantial individual variations in methylation changes, with some participants showing increases and others decreasing over time [35]. Factors potentially contributing to these discordant findings include differences in the brain regions studied, tissue processing methods, and detection techniques and protocols [15, 31]. Although these

inconsistencies suggest a complex relationship between global methylation changes and AD progression, they parallel findings in cancer studies where global hypomethylation and hypermethylation work together to simultaneously activate oncogenes and suppress tumor suppressor genes [36]. A similar interplay between localized hypomethylation and hypermethylation may contribute to AD progression and warrants further investigation.

GENE-SPECIFIC DNA METHYLATION CHANGES IN AGING AND AD

The progressive dysregulation of DNA methylation during aging is part of a broader phenomenon known as epigenetic drift. Epigenetic drift refers to the gradual and stochastic changes in epigenetic marks, especially DNA methylation, that accumulate with age [37]. This process is influenced by both intrinsic factors, such as errors in maintaining methylation patterns during DNA replication, and extrinsic factors, including environmental influences like diet, stress, and exposure to toxins. Although these changes originally seem non-directional and random [38, 39], growing evidence suggests that they tend to cluster at specific genomic loci, often in regions critical for cell identity and function [19, 40, 41]. Over time, epigenetic drift weakens cellular regulatory mechanisms, impacting gene expression, chromatin structure, and overall genomic stability. Epigenetic drift is increasingly recognized as a key contributor to various age-related diseases. In neurodegenerative diseases such as AD, specific methylation changes are observed in genes involved in neuronal survival and inflammation, likely due to age-related epigenetic drift (Table 1).

AD exists in both familial and sporadic forms. Familial AD represents 5–10% of cases and is typically early-onset (before age 65), primarily caused by mutations in three genes involved in the amyloid precursor protein (APP)-processing pathway: *APP, PSEN1,* and *PSEN2* [42, 43]. However, these mutations are rare in sporadic AD, despite the similar pathological characteristics in both forms. This suggests that mechanisms other than gene mutations may contribute to the dysfunction of these genes and their protein products. This hypothesis has been supported by several studies reporting hypomethylation in genes involved in the amyloidogenic pathway, including *APP, PSEN1,* and *BACE1* genes [44–46] Hypomethylation of these genes has been observed in both brain tissues [14, 44] and blood samples [45–47], boosting their expression and subsequently giving rise to the mass build-up of Aβ peptides, a hallmark of AD pathology.

The *MAPT* gene encodes the tau protein, which is crucial for stabilizing neurons' microtubules. In AD, tau proteins become abnormally phosphorylated and accumulated, forming neurofibrillary tangles (NFTs), one of the pathological hallmarks of the disease [27]. Several studies have shown that hypomethylation at specific CpG sites in the *MAPT* promoter region in AD-affected brains is associated with increased tau expression, suggesting a potential mechanism for tau upregulation [44, 48]. The *APOE* ε4 allele is the most significant genetic risk factor for AD. The ApoE protein is the main cholesterol carrier in maintaining, growing, and repairing neurons, and is usually highly expressed in the central nervous system [49]. Hypermethylation in the *APOE* promoter region has been observed in the prefrontal cortex of AD patients, consistent with reduced circulating ApoE levels in these patients [50, 51]. Similarly, a correlation between AD and increased

methylation levels at the *APOE* promoter has been found [52]. GSK3β is the kinase most frequently linked to the hyperphosphorylation of tau, a critical step in the aggregation and formation of NFTs. In the early stages of AD, DNA hypomethylation was observed in the promoter region of *GSK3B* in the prefrontal cortex, leading to elevated GSK3β expression in individuals with early AD [53].

While the amyloid and tau hypotheses have been central to AD research for decades, increasing evidence suggests that neuroinflammation plays a crucial role in AD pathogenesis [54]. Neuroinflammation refers to the activation of the brain's innate immune response, particularly involving glial cells such as microglia and astrocytes. In healthy brains, these glial cells help maintain homeostasis by clearing debris, regulating synaptic plasticity, and providing neurotrophic support. However, in AD and other neurodegenerative diseases, chronic activation of microglia and astrocytes leads to a prolonged inflammatory state, contributing to neuronal dysfunction and cell death [55]. Numerous studies have shown that neuroinflammation in AD is tightly regulated by epigenetic modifications, including DNA methylation [56]. For instance, the promoters of genes encoding pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 may become hypomethylated in response to chronic inflammation, leading to sustained expression and perpetuation of the inflammatory response. Conversely, hypermethylation of anti-inflammatory genes can suppress their expression, further skewing the balance towards a pro-inflammatory state [54]. The *TERM2* gene is expressed in microglia and participates in modulating the inflammatory response to amyloid plaques. Methylation changes in the *TREM2* promoter can affect its expression, influencing microglial activation and neuroinflammatory response in AD [57].

AD is characterized by cognitive decline, particularly memory impairment. Neuroplasticity is the brain's ability to modify its structure and function in response to experiences, environmental changes, and injury. It includes processes such as synaptic plasticity, dendritic branching, and neurogenesis. Synaptic plasticity, the ability of synapses to strengthen or weaken over time, is central to learning and memory. The capacity for neuroplasticity decreases with age, but remains an essential component of brain function. In healthy aging, the brain retains the ability to adapt and form new connections, though more slowly. In AD, neuroplasticity is significantly impaired, leading to the disruption of memory and other cognitive processes [58]. One of the earliest pathological changes in AD is synaptic dysfunction, which correlates strongly with cognitive decline. Synaptic loss, more than the presence of plaques or tangles, is considered one of the best predictors of memory impairment in AD [59]. Brain-derived neurotrophic factor (BDNF) is a neurotrophin that plays a crucial role in synaptic plasticity, neurogenesis, and neuronal survival. It is essential for learning, memory formation, and cognitive function, as it regulates synaptic growth, strengthens synaptic connections, and enhances long-term potentiation. BDNF exerts its effects through binding to the TrkB receptor, which activates intracellular signaling pathways such as the MAPK/ERK and PI3K/AKT pathways, which are important for neuronal survival and synaptic plasticity [60]. Several studies have reported significantly higher levels of DNA methylation in the *BDNF* promoter in the hippocampus and cortex, as well as peripheral blood samples from patients with AD [61, 62]. This reduction in BDNF levels is strongly correlated with synaptic loss, which is a key feature of AD pathology. Environmental factors such as stress, diet, and physical activity can influence

BDNF methylation. Chronic stress, which has been linked to an increased risk of AD, is associated with increased methylation of the *BDNF* gene and reduced *BDNF* expression. In contrast, physical activity, which is known to enhance *BDNF* expression and improve cognitive function, may counteract *BDNF* hypermethylation. Lifestyle interventions that reduce *BDNF* methylation and restore its expression could have protective effects on AD [63]. Aβ, the toxic protein that forms plaques in the brains of AD patients, has been shown to influence *BDNF* expression through epigenetic mechanisms. Aβ accumulation can induce hypermethylation of the *BDNF* gene, further reducing its expression and exacerbating synaptic dysfunction and cognitive impairment. This suggests a potential feedback loop in which Aβ pathology and *BDNF* dysregulation mutually reinforce each other, contributing to the progression of AD [64]. Another gene implicated in these processes is Sorbin and SH3 Domain Containing 3 (*SORBS3*), which has been found to be hypermethylated in the brains of AD patients and mouse models of AD [65, 66].

DNA METHYLATION DYNAMICS DURING AGING

Epigenetic factors may offer a more suitable explanation for the abnormalities observed in late-onset Alzheimer's disease (LOAD) than genetic factors, largely due to their influence on DNA methylation patterns throughout development. The epigenome, highly susceptible to dysregulation during critical life stages such as early embryonic development, neonatal periods, puberty, and particularly old age—which is the primary risk factor for AD—undergoes substantial shifts. A growing body of research has focused on understanding the cellular and molecular dimensions of aging, with epigenetic modifications identified as a key mechanism driving the deterioration of cellular functions associated with aging and related conditions. Notably, the gradual alterations in DNA methylation over time are thought to play a significant role in the onset of age-related diseases [23, 67–69].

Both hyper- and hypomethylation characterize this epigenetic drift and vary across the genome and among individuals of the same age. While epigenetic drift can lead to unpredictable differences in the methylomes of aging individuals, certain changes are associated with specific genetic regions, indicating that DNA methylation alterations are not entirely random but play a role in the aging process. Recent advances in high-throughput technologies, such as whole-genome bisulfite sequencing (WGBS) and DNA methylation arrays, have facilitated the study of age-specific DNA methylation patterns [70]. These changes primarily manifest as alterations in methylation levels across specific CpG sites in a certain pattern, rather than occurring randomly. These regions are known as age-associated differentially methylated positions (a-DMPs) or regions (a-DMRs) [71, 72]. Research indicates that with aging, methylation levels at these sites often trend towards an intermediate level with a reduced dynamic range. While some age-related DNA methylation changes are specific to certain tissues, there are also recurring patterns observed across multiple tissues and cell types including stem cells. These consistent methylation shifts often reflect systemic biological processes that contribute to aging and are linked to various age-related diseases [19, 73]. The exact causes and functional consequences of these methylation changes remain incompletely understood, as do their links to aging, longevity, and disease.

DNA METHYLATION CLOCK AND ACCELERATED AGING

These insights have led to the development of DNA methylation clocks, which use DNA methylation patterns in selected CpG sites to calculate an individual's biological age [74] [75]. While biological age and chronological age are strongly correlated, the difference between them may serve as a promising biomarker for assessing the risk of age-related diseases, such as type 2 diabetes, AD, and cardiovascular diseases [11, 12]. When an individual's biological age is significantly older than the chronological age, it indicates accelerated aging, a condition where the biological processes of aging advance more rapidly than expected. This phenomenon, often accompanied by shifts in DNA methylation patterns, correlates with increased vulnerability to various age-related diseases, poor health outcomes, higher mortality rates, and shorter life expectancy [11] [76]. Meanwhile, premature biological aging has also been observed in individuals infected by HIV [77, 78], or with reduced mental and physical fitness [79].

DNA methylation clocks have evolved through two generations (Table 2). The first generation was designed primarily to predict chronological age based on DNA methylation levels. Among these, the Horvath clock is widely validated across all cell types, which uses DNA methylation data from 353 CpG sites across the genome to estimate biological age [80]. The Hannum clock is based on DNA methylation data from 71 CpG sites and is specifically designed to use data from peripheral blood samples [39]. The Zhang DNA methylation clock adjusts for cellular composition to improve accuracy across different sample types [81]. Efforts are underway to develop clocks with fewer CpG sites to reduce costs and enhance efficiency, though these may be less reliable across multiple tissues.

Second-generation clocks, such as epiTOC [82], MiAge [83], PhenoAge [84], DNAmTL [85], GrimAge [86], and the Dunedin Pace of Ageing clock [87], build on the foundation of first-generation clocks but incorporate additional information related to age-related biological processes. These clocks factor in mitotic activity, telomere length, plasma biomarkers, organ functions, lifestyle, and clinical measures. As a result, they not only predict chronological age but also provide insights into an individual's risk of developing age-related diseases. The PhenoAge Clock, developed in 2018, represents a major step forward in epigenetic clock technology. Instead of merely predicting chronological age, PhenoAge estimates phenotypic age, taking into account both DNA methylation and clinical biomarkers associated with aging, such as glucose levels, C-reactive protein (CRP), and white blood cell count. This clock better captures age-related health decline and predicts the risk of disease and mortality [84]. The GrimAge Clock incorporates DNA methylation markers associated with risk factors for mortality, such as smoking pack-years known to affect lifespan. It predicts time-to-death and other age-related outcomes more accurately than earlier clocks [86]. More DNA methylation clocks have been developed using various training models, each capturing different aspects of disease and aging [81, 88, 89].

Epigenetic clocks have been explored as a tool to understand the relationship between biological aging and AD. Examinations of brain tissue from AD patients have reported that epigenetic age acceleration is associated with several key pathological features, including amyloid plaque deposition, tau pathology, neuroinflammation and neuronal loss [90–92].

Using the DNA methylation age (DNAm age) estimated by the Horvath clock, a study found that the prefrontal cortex of AD patients displayed significant epigenetic age acceleration, which was correlated with increased neurofibrillary tangle burden [93]. Other studies have shown that accelerated aging is associated with lower cognition, longitudinal cognitive decline, and reduced white matter integrity [93–95]. However, several other studies failed to find a significant link between Horvath DNAm age acceleration and cognitive function or brain volume, both in cross-sectional and longitudinal analyses [96, 97]. Similarly, DNAm age estimated by the Hannum clock has been linked to smaller hippocampal volume in one study [92], but did not show any significant associations with cognitive decline in another study [98].

Limited studies also examined the relationship between DNAm age estimated by the second-generation clocks and AD phenotypes. It has been shown that using the PhenoAge clock can detect associations between epigenetic age acceleration and risk of cognitive decline [99]. Individuals who exhibit accelerated GrimAge are more likely to experience cognitive deficits, particularly in memory, executive function, and processing speed [90, 100]. Although the initial results are promising, more extensive research is needed before a credible association can be established between DNAm aging and AD.

EPIGENETIC CHANGES IN OTHER AGE-RELATED DISEASES

Other age-related diseases like cardiovascular diseases (CVD) and Type 2 diabetes mellitus (T2DM) exhibit some similar patterns of epigenetic changes to AD, plausibly due to the shared effects of accelerated aging on the genome and cellular processes [101]. For example, aging is often accompanied by low-grade, chronic inflammation driven by immune system changes, cellular debris, and senescent cells that release inflammatory cytokines. This chronic inflammation affects DNA methylation of immune-related genes, making the body more prone to inflammatory responses, which underline AD, CVD, and T2DM [102–104]. In addition, aging is accompanied by dysregulation of proteostasis genes and a decline in the ability to maintain proteostasis, resulting in the accumulation of misfolded or aggregated proteins. Deposition of these proteins is a significant contributor to the onset and progression of not only AD but also other age-related diseases such as CVD and T2DM [105–107].

Aging also compromises autophagy, increases oxidative stress, and decreases mitochondrial function. Impaired mitochondria overproduce reactive oxygen species, leading to oxidative stress that damages proteins, lipids, and DNA. This oxidative burden disrupts proteostasis, overwhelming the cell’s ability to repair or degrade damaged proteins, which then accumulate, disrupting cellular functions. These damaged mitochondria and protein aggregates further impair autophagy, reducing the cell’s capacity to clear dysfunctional components and intensifying cellular stress. The resulting damage triggers chronic inflammation, creating a vicious cycle that perpetuates mitochondrial dysfunction.
The interplay between inflammation, mitochondrial dysfunction, proteostasis disruption, oxidative stress, and compromised autophagy forms a network of cellular stresses that accelerates aging and contributes to age-related diseases.

Understanding age-related pathology has led to studies identifying factors that accelerate or decelerate the DNA methylation aging clock. Key accelerators include chronic stress, poor diet, inflammation, smoking, alcohol use, sleep loss, and environmental toxins—all of which intensify cellular aging and damage. To slow the DNA methylation aging clock, strategies like a balanced diet, regular exercise, quality sleep, stress management, and toxin avoidance are beneficial; supplements such as NAD+ may also support healthy methylation patterns, slowing age-related declines (Figure 2).

POTENTIAL PITFALLS WHEN STUDYING DNA METHYLATION

The studies discussed above reveal key findings related to DNA methylation across various tissue and cell types. However, when studying DNA methylation in age-related diseases, researchers must be aware of several potential pitfalls that could affect the validity and interpretation of their results.

DNA methylation patterns are highly tissue-specific and, therefore may not be consistent between brain and blood. Furthermore, the brain is a heterogeneous organ. Different brain regions may have distinct methylation profiles. Within each region, various cell types may also differ in their methylation. Davies *et al.* examined methylomic variations across whole blood and six brain regions: inferior frontal gyrus, middle frontal gyrus, entorhinal cortex, superior temporal gyrus, visual cortex, and cerebellum. They found that tissue-specific differentially methylated regions exhibited significant variation across brain regions, with some overlap in blood, indicating the potential for using peripheral tissues in neurobiological research [108]. Studies of epigenetic clocks in neurodegenerative and neuropsychiatric disorders using brain tissues are limited by insufficient data on specific brain regions and cell types [109]. Meanwhile, computational methods, such as Houseman's cellular proportion estimates in blood and Guintivano's neuronal and non-neuronal proportion estimates, have been developed to address the variability in cell-type proportions across individuals [110, 111].

Techniques like single-cell bisulfite sequencing can be used to isolate individual cells, generate DNA methylation profiles at single-cell single-base pair resolution, and identify cell type-specific patterns. Using these techniques, researchers may study cell-type-specific epigenetic alterations associated with a given disease phenotype, capturing the heterogeneity within complex tissues. After identifying significant epigenetic changes, researchers can perform targeted validation using cell sorting techniques (e.g., fluorescence-activated cell sorting) to capture cells of specific types and perform validation assays on these sorted cells to confirm findings, ensuring that observed changes are relevant in the exact cells impacted by the disease [112–115]. For example, Luo *et al.,* mapped methylomes of single neuronal nuclei from mouse and human frontal cortex, revealing unique methylation patterns and regulatory elements that drive brain cell diversity [112]. Liu *et al.,* examined the epigenomes of mouse brain cells using single-nucleus DNA methylation sequencing on over 100,000 nuclei from 45 regions of the mouse cortex. They identified 161 cell clusters with distinct methylation patterns, revealing the regulatory landscape behind neuronal diversity. Notably, the DNA methylation landscape of excitatory neurons in the cortex and hippocampus varied continuously along spatial gradients. An artificial neural network was then built to predict

cell-type identity and map enhancer-gene interactions in the mouse brain by integrating DNA methylomes with chromatin accessibility [113].

However, the high cost of these techniques creates an obstacle for large-scale study. Future research needs to focus on cross-tissue study designs to identify differentially methylated positions/regions that are common across multiple cell types, such as brain tissue and peripheral blood, to uncover shared correlations.

For degenerative brain disorders, such as AD, brain tissue samples are indispensable to study the disease mechanisms. Since obtaining brain tissue from living patients is nearly impossible, post-mortem samples are often used instead. However, these samples are subject to varying levels of degradation depending on the post-mortem interval, the time elapsed between the individual's death and the preservation of the sample. This variability can impact the quality of the tissue and influence the results of studies, potentially introducing artifacts or inconsistencies in the analysis of DNA methylation and other molecular markers. It may introduce confounding effects on methylation patterns due to the uncertainty about whether the observed epigenetic patterns are caused by the disease process or by factors such as the cause of death, tissue pH, and the premortem agonal state [116]. For example, the Karlsson group investigates the impact of post-mortem intervals on DNA modifications 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) in rat cerebellum tissues. Their findings indicate that while levels of 5mC decrease, levels of 5hmC increase with longer post-mortem intervals [117]. Jarmasz *et al.* found that DNA methylation remained stable for up to 72 hours post-mortem, suggesting a limit on sample handling time [118]. Researchers need to have strict control for post-mortem intervals and use fresh or well-preserved samples where possible.

Because of the challenges associated with DNA methylation analysis in brain tissue, studying DNA methylation using peripheral cells such as blood in AD patients has gained interest as a potential non-invasive biomarker approach. The use of peripheral tissues as biomarkers for diseases affecting specific organs is often debated, given that peripheral cells may not accurately reflect central disease processes. However, DNA methylation changes in blood cells are already used to estimate "epigenetic age," and accelerated aging could be associated with the progression of AD phenotype. Existing data show that measuring epigenetic age acceleration in blood samples could provide a novel biomarker for disease risk or progression. Furthermore, while blood and brain tissues are different, some DNA methylation changes in blood cells may mirror or correlate with those in the brain. This is particularly relevant for genes involved in immune response or systemic inflammation, which are implicated in AD [119]. Furthermore, specific methylation markers, such as those in the *MAPT* and *PSEN1* genes, have been identified in both the blood and brain tissues of AD patients. These findings suggest that methylation changes in blood could serve as peripheral biomarkers for neurodegenerative processes occurring in the central nervous system [50]. However, more research is needed before we have sufficient evidence to suggest that AD-related epigenetic modifications in the brain can be reflected in the periphery.

Whole blood consists of a variety of cell types, such as neutrophils, lymphocytes, monocytes, eosinophils, and others. The distribution of these cells can be influenced by numerous factors, including infections (bacterial or viral), inflammation, diet, stress, medications, and environmental exposures. As a result, it is essential to account for these varying cell proportions in any analysis, as they can introduce confounding variables that may obscure true biological signals or lead to inaccurate conclusions. Controlling for cell type composition is therefore critical to ensuring the reliability and validity of findings in blood-based studies.

Lastly, peripheral blood is relatively easy to collect, making it ideal for repeated sampling over time. This also allows for longitudinal studies that can track changes in methylation throughout the progression of AD and enable earlier diagnosis before clinical symptoms become evident.

CONCLUSION

Over the past decade, DNA methylation has been linked to age-related diseases, including cancer, heart disease, and AD. Numerous studies have revealed evidence of age-related changes in methylation patterns by exploring both global and site-specific CpG DNA methylation in aging and AD. Identifying AD-specific methylation patterns in accessible tissues like peripheral blood could enable the development of non-invasive biomarkers for early diagnosis, disease monitoring, and predicting progression. Furthermore, DNA methylation changes are potentially reversible, and understanding methylation changes in AD could lead to novel therapeutic strategies aimed at modifying disease-related epigenetic marks. Recently, researchers have identified epigenetic clocks. Emerging research indicates that patients with age-related diseases or phenotypes often exhibit accelerated aging as measured by these clocks compared to healthy individuals. As a result, there is increasing interest in using epigenetic clocks, especially in easily accessible tissues such as peripheral blood, as biomarkers for detecting or even predicting aging and age-related diseases. Although the research on DNA methylation changes in AD holds promise for improving diagnosis, treatment, and prevention strategies, there are many challenges such as tissue specificity, availability, and cell-type heterogeneity. Addressing these challenges is essential to advance our understanding of how DNA methylation contributes to AD.

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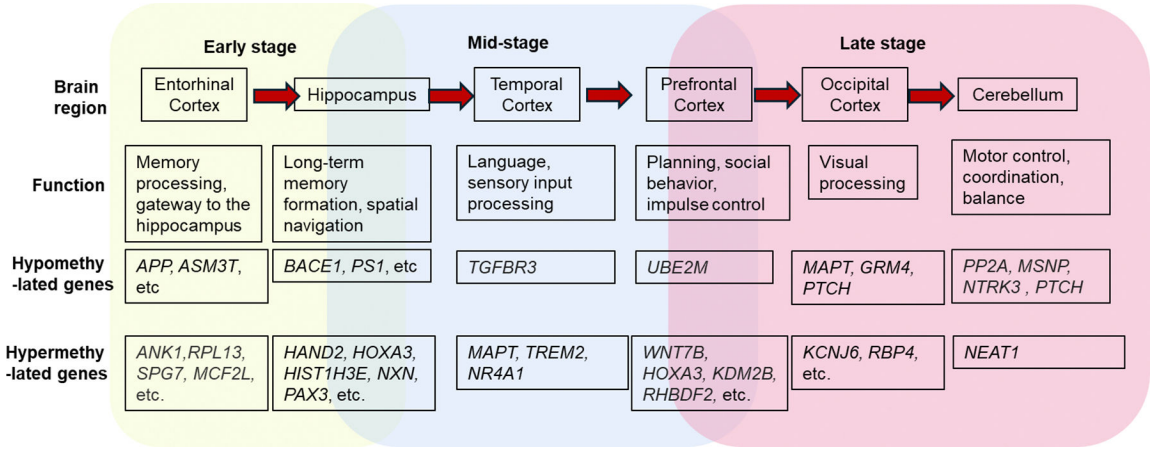

Figure 1.

AD progresses through specific brain regions, leading to functional decline and varied DNA methylation changes. The early stages involve hypermethylation of genes like *ANK1* and *MAPT* in memory-related regions such as the entorhinal cortex and hippocampus. In mid-stages, sensory and cognitive regions like the temporal and frontal cortices show mixed methylation patterns, affecting genes such as *SORL1, APP,* and *TREM2.* In late stages, regions linked to executive function and sensory processing, such as the prefrontal, parietal, and occipital cortices, show predominant global hypermethylation, with specific genes like *PSEN1* and *CLU* being highly methylated.

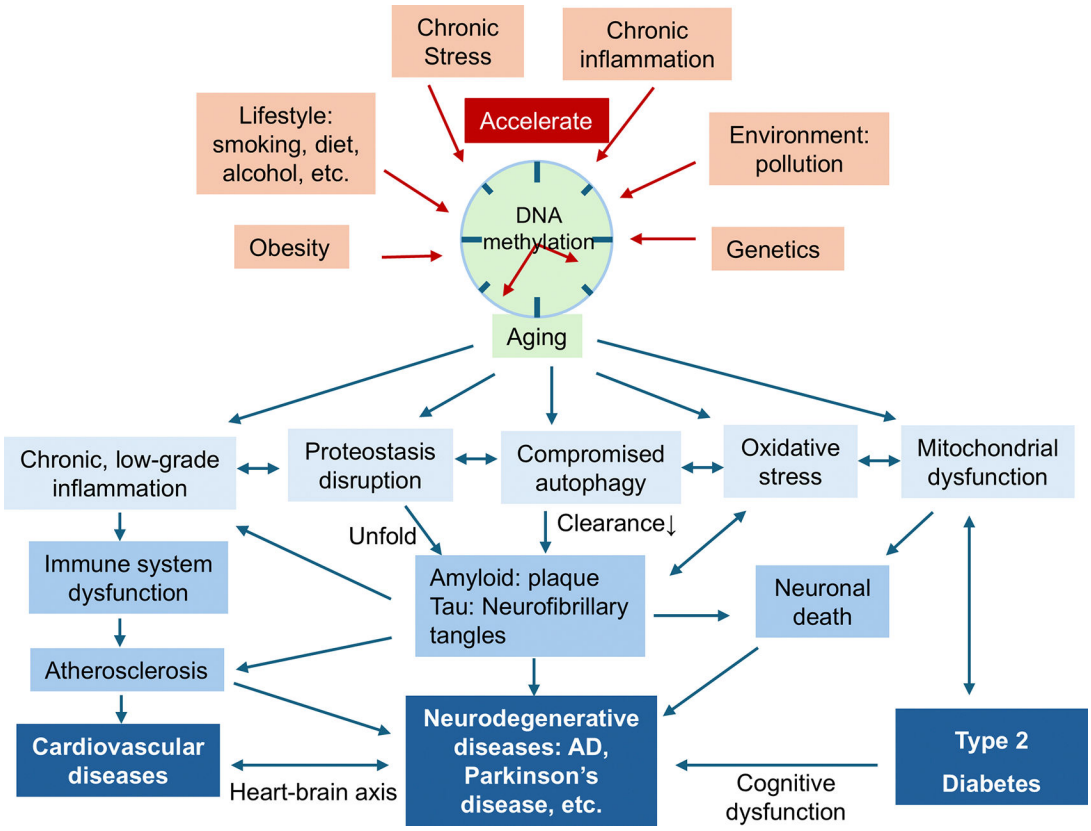

Figure 2.

Relationship between age-related diseases and regulatory factors. Aging increases chronic inflammation, disrupts proteostasis, compromises autophagy, elevates oxidative stress, and decreases mitochondrial function. The interplay among these changes creates a network of cellular stresses that contributes to age-related diseases, including cardiovascular disease, neurodegenerative diseases, Type 2 diabetes, and others. Factors such as obesity, lifestyle, chronic stress, and genetics further accelerate the DNA methylation aging clock.

Table 1.

Examples of important genes showed DNA methylation changes with age and in Alzheimer's disease.

Gene	Brain Region	Gene Function	Methylation in AD	Related Pathway	Age Related?	Clinical Correlation
ABCA7	Prefrontal Cortex	Lipid transport, amyloid-beta clearance	Hyper	Lipid metabolism, amyloid clearance	Yes	Increased AD risk, lipid dysregulation, amyloid accumulation
APOE (ε4)	Hippocampus, Temporal Cortex	Lipid transport, modulates amyloid-beta clearance	Hypo (ε4 allele)	Lipid metabolism, amyloid clearance	Yes	Increased risk of AD, accelerated progression in APOE ε4 carriers
APP	Temporal Cortex, Hippocampus	Amyloid precursor protein, involved in amyloid-beta production	Hypo	Amyloid-beta production, plaque formation	Yes	Increased amyloid-beta production, amyloid plaques
BACE1	Temporal Cortex, Hippocampus	Beta-secretase enzyme, involved in amyloid-beta cleavage	Hypo	Amyloid-beta production	Yes	Higher amyloid-beta levels, increased AD risk
BDNF	Hippocampus, Cortex	Neuron survival, differentiation, synaptic plasticity, and neurogenesis	Hyper	TrkB, MAPK/ERK, and PI3K/AKT	Yes	Cognitive decline in learning and memory.
MAPT (Tau)	Prefrontal Cortex, Temporal Cortex	Microtubule-associated protein, stabilizes microtubules	Hyper	Tau phosphorylation, tangle formation	Yes	Increased neurofibrillary tangles, tau pathology
NR3C1	Hippocampus, Temporal Cortex	Glucocorticoid receptor, involved in stress response	Hyper	Stress response, HPA axis	Yes	Linked to increased AD risk through chronic stress and inflammation
PSEN1	Temporal Cortex	Part of gamma-secretase complex, involved in amyloid processing	Hyper	Amyloid-beta processing	No	Early-onset AD mutations, cognitive decline
PTEN	Hippocampus	Tumor suppressor, regulates cell survival	Hyper	PI3K/AKT signaling, apoptosis	Yes	Impaired synaptic plasticity, cognitive decline
SOD2	Prefrontal Cortex	Antioxidant enzyme, reduces oxidative stress	Hyper	Oxidative stress, ROS management	Yes	Enhanced oxidative stress, linked to neurodegeneration
TREM2	Hippocampus, Temporal Cortex	Microglial activation, immune response regulator	Hyper	Inflammation, immune response	No	Impaired microglial response, inflammation, neurodegeneration

Table 2.

Difference of 1st and 2nd generation DNA methylation clocks.

Clock	Generation	Developer	Tissue Types	Number of CpG Sites	Prediction Accuracy	Main Use	Special Features
Horvath's Clock	1st	Horvath (2013)	Multiple tissues (pan-tissue)	353	High across various tissues	Estimating biological age across tissues	Pan-tissue, widely applicable
Hannum's Clock	1st	Hannum (2013)	Whole blood	71	High for blood	Estimating biological age from blood	Blood-specific model
EpiTOC	1st	Teschendorff (2016)	Blood, tissues (epigenetic aging)	385	High for cell division rate	Aging in proliferating tissues, cancer research	Focuses on cell division rate as a measure of age
PhenoAge Clock	2nd	Levine (2018)	Blood	513	High correlation with mortality	Predicting morbidity and mortality risk	Incorporates clinical biomarkers for healthspan estimation
Skin & Blood Clock	2nd	Horvath (2018)	Skin and blood	391	High for skin and blood	Estimating biological age in skin and blood tissues	More accurate for specific tissues (skin and blood)
Zhang's Clock	1st	Zhang (2019)	Blood	10	Moderate	Aging research, mortality prediction	Simplified model with fewer CpGs, mortality predictor
GrimAge Clock	2nd	Horvath (2019)	Blood	1030	Strong association with lifespan	Predicting lifespan, healthspan, and disease	Uses DNA methylation + plasma proteins for lifespan estimation
MiAge	2nd	Hongmei Nan (2019)	Mitochondria-related	36 CpG regions	High, linked to mitochondrial function	Estimating aging based on mitochondrial DNA methylation	Focuses on mitochondrial health and aging
DNAmTL Clock	2nd	Lu (2019)	Blood	140	Telomere length correlation	Predicting telomere length	Predicts telomere length via DNA methylation