Review

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An Epigenetic Manifestation of Alzheimer's Disease: DNA Methylation

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Abstract

Alzheimer's disease (AD), the most common form of dementia, has a complex pathogenesis. The number of AD patients has increased in recent years due to population aging, while a trend toward a younger age of onset has arisen, imposing a substantial burden on society and families, and garnering extensive attention. DNA methylation has recently been revealed to play an important role in AD onset and progression. DNA methylation is a critical mechanism regulating gene expression, and alterations in this mechanism dysregulate gene expression and disrupt important pathways, including oxidative stress responses, inflammatory reactions, and protein degradation processes, eventually resulting in disease. Studies have revealed widespread changes in AD patients' DNA methylation in the peripheral blood and brain tissues, affecting multiple signaling pathways and severely impacting neuronal cell and synaptic functions. This review summarizes the role of DNA methylation in the pathogenesis of AD, aiming to provide a theoretical basis for its early prevention and treatment.

Keywords

Alzheimer's disease; DNA methylation; oxidative stress response; inflammatory reaction

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive deterioration in cognitive function, memory loss, a decrease in reasoning ability, and difficulty carrying out daily activities [1]. With the accelerating population aging trend, AD incidence is gradually increasing, posing a major challenge to public health [2]. AD also substantially impacts national healthcare financing and socioeconomic status [3]. Therefore, exploring effective mechanisms for treating or alleviating AD occurrence and progression has become a research focus.

Research indicates that pathological physiological alterations, such as aberrant accumulation of beta-amyloid within neurons and abnormal phosphorylation of the tau protein, are closely associated with AD onset [4]. The aberrant expression of these biomarkers mediates structural and functional changes within the neuronal architecture of the human brain, thereby inducing further deterioration of patients' cognitive functions. In addition, inflammatory responses play a significant role in AD pathogenesis [5].

DNA methylation, an epigenetic modification characterized by the addition of methyl groups to DNA molecules, plays a pivotal role in regulating gene expression. This process is instrumental in maintaining genetic stability, modulating cellular development, and adapting to environmental changes [6,7]. Aberrations in DNA methylation patterns, which primarily occur at CpG dinucleotide sites, have been implicated in the onset and progression of many diseases [8,9].

Despite significant research advancements over the past several decades, the precise etiology and pathogenic mechanisms of AD remain incompletely understood. Historical investigations have predominantly focused on the

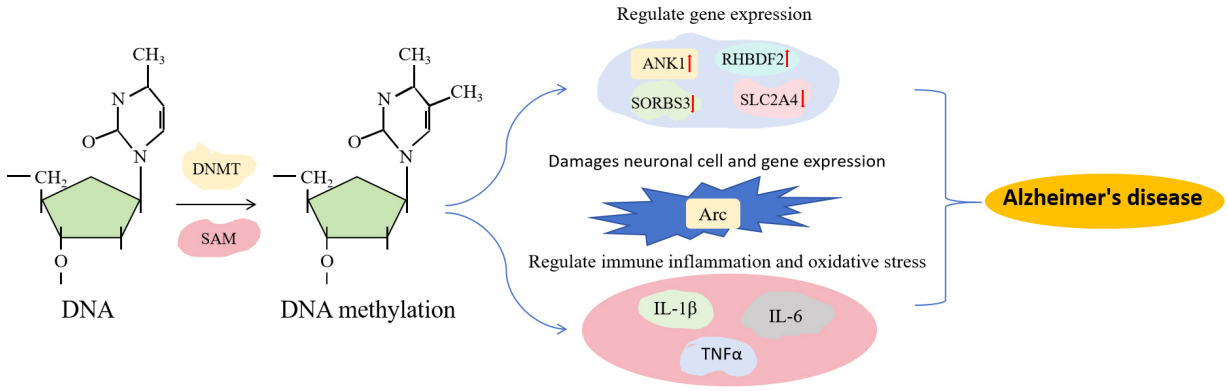

Fig. 1. Diagram of the mechanism that DNA methylation in Alzheimer's disease. DNA in cells is methylated under the combined action of DNA methyltransferase (DNMT) and S-adenosyl methionine (SAM), which regulates gene expression, damages neuronal cells, and regulates immune inflammation and oxidative stress, thus leading to the occurrence of Alzheimer's disease. SORBS3, sorbin and SH3 domain containing 3; ANK1, Ankyrin 1; RHBDF2, rhomboid 5 homolog 2; SLC2A4, solute carrier family 2A; IL-1β, interleukin 1β; IL-6, interleukin 6; TNFα, tumor necrosis factor α; Arc, activity regulated cytoskeleton associated protein. This image is an original image, its production software is WPS Office (12.1.0.16388, Kingsoft Corporation, Beijing, China).

genetic and neurobiological aspects of AD; however, a growing body of recent evidence suggests that epigenetic modifications, particularly changes in DNA methylation, may play a crucial role in the pathogenesis of this disease [10–12]. Hence, this review aims to shed light on how DNA methylation contributes to AD development and progression, offers new perspectives for elucidating the pathogenic mechanisms of this disease, and provides a scientific basis for developing more effective therapeutic targets and preventive strategies. The relationship between DNA methylation and the development of AD in Fig. 1.

Basic Mechanism of DNA Methylation

Definition and Process of DNA Methylation

DNA methylation is a form of DNA chemical modification that can alter gene expression without changing the DNA sequence. DNA methylation involves the covalent attachment of a methyl group to the 5th carbon position of cytosine within CpG dinucleotides in the genome and is catalyzed by DNA methyltransferases [13]. Extensive research has demonstrated that DNA methylation can induce changes in chromatin structure, DNA conformation, DNA stability, and interactions between DNA and proteins, thereby regulating gene expression [14,15]. DNA methylation is among the earliest discovered and most thoroughly studied epigenetic regulatory mechanisms. Broadly, DNA methylation refers to a chemical modification process in which a specific base in the DNA sequence acquires a methyl group through a covalent bond under the catalysis of DNA methyltransferases (DNMTs) using S-adenosyl methionine (SAM) as the methyl donor [16]. This methylation can occur at several sites, including the C-5 position of cytosine, the N-6 position of adenine, and the G-7 position of guanine. However, in general, DNA methylation primarily refers to the methylation process at the 5th carbon atom of cytosine within CpG dinucleotides, resulting in 5-methylcytosine (5-mC), which is the predominant form of DNA methylation in eukaryotes, such as plants and animals, and the only form identified in mammalian DNA methylation [17,18]. DNA methylation is a relatively stable modification state and can be inherited by progeny DNA during the replication process under the action of DNA methyltransferases, constituting a significant epigenetic mechanism.

Biological Function of DNA Methylation

DNA methylation serves multiple critical functions in biology. First, it plays a pivotal role in maintaining genomic stability and integrity [6]. By introducing methyl groups into the promoter regions of genes, DNA methylation can inhibit transcription, thereby regulating gene expression levels [19,20]. Second, DNA methylation underscores heterogeneity across different genomic regions, facilitating differential gene functions among cell types. Moreover, DNA methylation regulates biological processes such as chromosomal structure and cell differentiation [21]. The hypermethylation or hypomethylation of CpG sites can serve as a dynamic marker of gene–environment interactions over time, with changes in DNA methylation levels influencing gene expression through alterations in chromatin structure [22].

Relationship between DNA Methylation and Gene Expression

DNA methylation, a critical epigenetic regulatory mechanism, primarily involves the addition of methyl groups to cytosine at CpG sites to form 5-methylcytosine [23–25]. Extensive research has demonstrated that DNA methylation can influence gene transcription and expression, thereby participating in various biological processes [19,20]. The relationship between DNA methylation and gene expression is complex and precise. In the human genome, CpG islands, which are rich in CpG sites, are predominantly located in promoter regions or in the first exons of genes [26]. Methylation of these CpG islands initiates a gene silencing process: first, by interfering with the binding of transcription factors to promoter sequences, thus inhibiting the assembly of the transcription initiation complex; second, by recruiting methyl-CpG binding proteins and other corepressor factors, altering chromatin conformation and inhibiting gene transcription; and third, by affecting the cutting activity of DNA topoisomerase II, reducing DNA accessibility [27]. Additionally, methylation in low CpG density areas within the gene body primarily stabilizes gene expression by influencing histone lysine methylation and nucleosome remodeling [28]. These mechanisms illustrate how DNA methylation alters chromatin states, thereby repressing or activating gene transcription.

In recent years, the integration of large-scale methylation and gene expression data through multiomics analyses has confirmed that DNA methylation extensively influences transcriptional levels. However, the quantitative relationship between DNA methylation and gene expression is complex and potentially affected by genomic location and the contextual sequence. Moreover, this quantitative association exhibits variability across different tissues and cell types.

Pathophysiology of AD

Amyloid-beta Protein Aggregation

Amyloid-beta protein (Aβ) is a peptide fragment derived from the hydrolytic cleavage of amyloid precursor protein (APP), a product of the amyloidogenic pathway of APP processing [29,30]. Under normal physiological conditions, Aβ produced in the body can be degraded by insulin-degrading enzymes and neprilysin and is thereby expelled from the body through physiological activities [31]. However, nondegraded Aβ can evade detection by the blood–brain barrier (BBB) and enter the circulatory system [32]. Intrinsically, Aβ monomers exert certain physiological activities that protect mature neurons from excitotoxic death and can modulate voltage-gated potassium channels on the cell membrane to regulate potassium ion flux [33,34]. Conversely, aggregated Aβ forms a plethora of neurotoxins, impeding normal neuronal signal transduction and disrupting intercellular communication, thereby inducing aberrant neuronal apoptosis or death and ultimately leading to neural network dysfunction [35].

Neurofibrillary Tangles

Neurofibrillary tangles are pathological changes in the cortical neurons of AD patients and are one of the major pathological hallmarks of AD [36–38]. The main component of neurofibrillary tangles is an abnormally phosphorylated protein that aggregates into paired helical filaments (PHFs). This protein is commonly known as tau. Tau is a normal phosphoprotein in the human brain and is located in axons and neuronal cell bodies [39]. The tau gene is located on chromosome 17q21 in humans, and alternative splicing produces 6 isoforms ranging from 351 to 441 amino acids in length. Under certain stimuli, tau undergoes abnormal hyperphosphorylation, leading to conformational changes, the aggregation of neurofibrils (i.e., neurofibrillary tangles), and the disruption of the neuronal cytoskeleton. These changes impair normal neuronal function [40]. Neurofibrillary tangles further cause axonal and dendritic retraction and other degenerative changes, ultimately leading to neuronal death [41]. Neurofibrillary tangles are very common in AD patients, and study has shown that they are closely associated with the pathophysiological progression of AD [42]. Thus, neurofibrillary tangles have become an important focus in the study and treatment of this disease.

Neuronal Injury and Cell Death

Neurons form the basic structural and functional units of the central and peripheral nervous systems; thus, neuronal injury and death are common pathological features of various neurological diseases [43,44]. The mechanisms underlying neuronal damage and death are current neuroscience research foci and challenges. Neuronal injury can be caused by various factors, including external mechanical damage, viral/bacterial infections, neurodegenerative diseases, toxins, and drug effects [45–47]. These insults can directly or indirectly lead to a series of intracellular and extracellular pathological changes, such as cytoskeletal collapse, metabolic disturbances, oxidative stress, inflammatory factor release, and calcium homeostasis dysfunction. Programmed cell death occurs if the damage exceeds a certain threshold and the neuron cannot repair itself. The cur-

rently recognized pathways of neuronal death mainly include apoptosis, autophagy, and necrosis [48,49].

Neuronal damage is the final outcome of AD progression. Aβ aggregation and neurofibrillary tangle formation disrupt normal neuronal function, leading to gradual cell death. Neurodegeneration occurs in specific brain regions, especially areas involved in learning, memory, and other cognitive functions, manifesting as significant neuronal loss and tissue damage [50,51]. Further neuronal death causes brain atrophy and shrinkage. Neuronal injury and cell death are the core mechanisms underlying cognitive and behavioral impairments in AD. Therefore, understanding and intervening in neuronal damage and cell death processes is critical for developing therapeutic strategies for AD.

In summary, the interplay of these pathological changes leads to the loss of neuronal function and abnormal structural alterations in the AD brain. These findings provide key clues for identifying therapeutic strategies targeting these pathological hallmarks.

DNA Methylation and Pathogenesis of AD

Growing evidence suggests that aberrant DNA methylation may be implicated in AD pathogenesis. Study has shown that, compared to the myelin methylation levels in normal controls, total myelin methylation levels decrease in the brain tissues of AD patients [52]. In addition, the methylation status of several key AD-related genes, such as Peroxisome Proliferator-activated Receptor gamma Coactivator 1-alpha (*PPARGC1A*) and sorbin and SH3 domain containing 3 (*SORBS3*), is also altered in AD patients [53,54]. These genes are involved in various biological processes, including metabolism, inflammation, cholesterol homeostasis, and microtubule stability. Changes in the methylation status of these genes may affect their expression and activity, ultimately contributing to AD development.

DNA Methylation Mediates the Dysregulation of Related Gene Expression, Leading to AD

DNA methylation is an important epigenetic mechanism that mainly occurs at CpG sites and regulates gene transcription by remodeling chromatin conformation [55]. Extensive research has revealed widespread alterations in DNA methylation patterns in the brain tissues of AD patients [56]. These differentially methylated genes are involved in AD-related pathological processes, including neurodevelopment, neuroplasticity, inflammatory immune responses, calcium homeostasis, cholesterol metabolism, and myelin metabolism. For example, the methylation levels of *SORBS3*, solute carrier family 2A (*SLC2A4*), and other genes decrease, while those of Ankyrin 1 (*ANK1*), rhomboid 5 homolog 2 (*RHBDF2*), and other genes increase [54]. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses have indicated that these dysregulated methylated genes are enriched in pathways related to synaptic transmission, long-term potentiation, and psychiatric disorders [57].

DNA hypomethylation has been strongly correlated with amyloid-β load, neuritic plaques, and diffuse plaques [58]. De Jager *et al.* [59] reported 71 CpG sites with methylation levels associated with AD pathology out of 415,848 sites analyzed, including CpGs in the ATP binding cassette subfamily A member 7 (*ABCA7*) and bridging integrator 1 (*BIN1*) gene regions, which harbor AD susceptibility variants. Other differentially methylated CpG sites near genes implicated in AD include *ANK1*, cadherin 23 (*CDH23*), disco interacting protein 2 homolog A (*DIP2A*), *RHBDF2*, ribosomal protein L13 (*RPL13*), *serinf1*, and *serinf2*. DNA methylation changes in AD susceptibility genes may occur before symptom onset and could represent an early disease characteristic. Hypomethylation of the APP and apolipoprotein E (APOE) promoters has been observed in some AD patient studies [60,61].

Neprilysin (NEP) is an important Aβ-degrading enzyme that has decreased expression in the AD brain. *In vitro* evidence has confirmed that Aβ increases NEP DNA methylation, thereby suppressing NEP mRNA and protein expression [62]. This finding may partially explain the ineffective Aβ clearance observed as AD progresses.

DNA methylation-mediated regulation of specific genes is a key molecular mechanism in AD. Highly methylated genomic regions are often associated with gene silencing, which may decrease the expression of AD-related genes in patients. Studies have revealed DNA methylation level changes resulting in gene upregulation or downregulation at the promoter regions of AD-related genes such as *APP*, presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), and microtubule associated protein tau (*MAPT*) [61,62]. DNA methylation is catalyzed by DNA methyltransferases (DNMTs), which add methyl groups to cytosine residues [63]. In contrast, DNA demethylases (TETs) can remove these methyl groups [64]. This balance is potentially disrupted in AD, resulting in aberrant methylation states of certain genes that impact their normal functions. DNA methylation might affect cell signaling pathways involving phosphorylation states, particularly those involving the tau pro-

tein. This influence may lead to abnormal tau aggregation and neurofibrillary tangle formation, accelerating disease progression. In summary, aberrant DNA methylation is a key process affecting neuronal biological functions in AD pathogenesis.

DNA Methylation Damages Neuronal Survival Networks in AD Patients

In recent years, the role of epigenetics in disease pathogenesis has received considerable attention. DNA methylation, a phenomenon of epigenetic modification, has been extensively studied. Research has revealed that DNA methylation plays an important role in the synaptic plasticity of neurons. Changes in DNA methylation may disrupt synaptic function and subsequently cause neuronal injury by mediating synapse formation and the expression of related genes [65]. Study has indicated that DNA methylation plays a key regulatory role in neuronal development and maturation [66]. DNA methylation increases AD risk by affecting normal neuronal development. It is also involved in regulating the expression of memory-related genes, disrupting memory formation and maintenance in the human brain, leading to cognitive decline in AD patients. Research has shown increased DNA methylation at the memory-associated gene *Arc* (activity regulated cytoskeleton associated protein) promoter region in AD mouse models, inhibiting its expression [67]. The aforementioned molecular mechanism might underlie early memory deficits in AD.

Abnormal DNA methylation is widespread in the brain tissues of AD patients throughout disease progression. Studies have shown that hypermethylation occurs at promoter regions of key neuronal survival and apoptosis genes, including the antiapoptotic factors brain derived neurotrophic factor (*BDNF*), B cell leukemia 2 (*Bcl-2*), and nerve growth factor (*NGF*), in AD patient brains, with corresponding decreases in downstream gene expression, reduced antiapoptotic protein synthesis, and impaired neuronal survival signaling networks [68–70].

DNA Methylation Mediates AD Development by Regulating Immune Inflammation and Oxidative Stress

DNA methylation potentially contributes to AD pathogenesis by regulating immune and inflammatory responses. Study has shown that changes in the DNA methylation of inflammation-related genes may lead to overactivation of inflammatory responses, exacerbating neuronal injury and pathological characteristic development [71]. Oxidative stress can induce alterations in DNA methylation levels; conversely, aberrant DNA methylation may cause antioxidant system dysfunction [72]. These interactions may play a role in AD pathogenesis, especially in cell damage and death processes. In recent years, increasing evidence has indicated a complex interplay between DNA methylation changes, neuroinflammation, and oxidative stress [73]. These processes may form a vicious cycle that jointly promotes AD onset and progression [73]. Research has shown that Aβ peptide deposition activates microglia and neuroinflammation, stimulating microglia to release inflammatory factors, including interleukin (IL)-1β, IL-6, and tumor necrosis factor α (TNFα), which can further increase the expression and activity of DNA methyltransferases (DNMTs) [74]. DNMT-mediated aberrant DNA methylation inhibits the expression of antioxidant and antiapoptotic genes, aggravating oxidative stress and cell apoptosis. Additionally, DNMT inhibitors can significantly reduce Aβ-induced inflammatory responses and oxidative damage [75]. These findings suggest that DNA methylation acts as an upstream event, forming a positive feedback loop with downstream inflammation and oxidative stress, damaging AD neurons and promoting irreversible disease progression.

Association of DNA Methylation with AD Clinical Features

Study has shown that DNA methylation patterns in the peripheral blood or cerebrospinal fluid of AD patients are significantly correlated with clinical symptoms and cognitive changes [76]. For example, increased methylation levels in promoter regions of antiapoptotic, synaptic plasticity, and neurotrophic genes in the hippocampus positively correlate with learning and memory impairments and decreased daily living abilities in AD patients [77]. The resulting gene expression inhibition potentially represents the molecular basis for neuronal functional deficits [77], laying the foundation for developing novel AD biomarkers and drug targets.

Furthermore, DNA methylation analysis of peripheral blood leukocytes can effectively reflect changes in the clinical features of AD patients. Study has utilized machine learning and deep learning algorithms to achieve over 80% diagnostic accuracy for AD by integrating and analyzing data from specific methylation sites [78]. This approach also enables the dynamic monitoring of disease progression. In summary, DNA methylation profiles show substantial potential for the early detection and precise diagnosis of AD. However, research in this field is still in its infancy, and specific molecular mechanisms need to be further elucidated.

Clinical Significance and Potential Application

DNA Methylation as a Biomarker for AD

AD is a common neurodegenerative disorder mainly characterized by progressive cognitive impairment and various neuropsychiatric symptoms [79]. With the aging of the global population, AD has become an important public health issue. Due to the complex pathogenesis of AD and the lack of specific diagnostic biomarkers, most patients are not diagnosed until symptoms become apparent, and the optimal treatment window is missed. Therefore, there is an urgent need to discover novel AD biomarkers, especially for early disease stages.

In recent years, significant alterations in DNA methylation patterns have been detected in AD patients' brain tissues and peripheral blood. DNA methylation is an important epigenetic regulatory mechanism, and abnormalities in DNA methylation are associated with the pathogenesis of various diseases. The application of high-throughput detection technologies has identified numerous AD-associated differentially methylated sites. For example, the methylation levels of the *ANK1*, *RHBDF2*, and *RPL13* genes increase in the blood and brain tissues of AD patients, while the methylation levels of *SORBS3*, *SLC2A4*, and other genes decrease [59,80,81]. These differentially methylated genes are involved in critical biological processes, including neurodevelopment, inflammatory immune responses, glucose metabolism, and cholesterol metabolism. Epigenetic changes potentially influence the role of related processes in AD pathogenesis. Moreover, study has shown that classification models constructed according to these differential methylation sites can serve as diagnostic biomarkers for AD [82], with efficacy surpassing known genetic susceptibility factors, such as apolipoprotein E (APOE) ε4 [83]. In summary, DNA methylation is a potential biomarker in AD research. Detecting DNA methylation patterns in blood or brain tissues might enable early AD diagnosis and risk prediction.

Repeutic Potential of DNA Methylation

DNA methylation is an important epigenetic regulatory mechanism that controls normal cellular physiological processes by influencing gene expression activation or silencing. In recent years, abundant research has demonstrated abnormal alterations in DNA methylation patterns in various diseases [84–86]. Thus, epigenetic therapy targeting DNA methylation has become a popular and new research direction.

DNA methylation-related drugs evaluated in clinical trials mainly include DNMT inhibitors and histone demethylase [homocysteine S-methyltransferase 1 (HMT), lysine demethylase (KDM)] inhibitors. Demethylases, such as lysine demethylase 6A (KDM6A) and lysine demethylase 6B (KDM6B), are associated with various diseases, including blood and solid tumors and autoimmune and inflammatory diseases, and can inhibit proliferation, induce apoptosis, promote differentiation, and increase sensitivity to currently used chemotherapy agents [87]. Li H *et al.* [88] showed that N6-methyladenine (6 mA) is a methylated adenine residue, and under the catalysis of the demethylase alkB homolog 1 (ALKBH1), 6 mA is oxidized by Fe^{2+}, O_2, and α-KG to 6 mA intermediates. Then, at 6 mA, adenine and formalin are spontaneously degraded without the catalysis of demethylases [88]. Additionally, in the field of neurodegenerative diseases, some specific demethylase inhibitors have shown potential for treating AD and Parkinson's disease.

In summary, epigenetic therapy targeting DNA methylation has tremendous application potential and will greatly promote the prevention and treatment of various diseases.

Conclusion

DNA methylation is an important epigenetic modification that plays a key role in AD pathogenesis. Abnormal DNA methylation patterns of specific genes are present in the brain tissue and peripheral blood of AD patients, and these abnormal methylation patterns potentially alter AD-related gene expression, affecting neuronal survival, synaptic plasticity, and other important biological processes [89]. Tingting Pi *et al.* [90] showed that the environmental factor Hcy might affect AD via the DNA methylation pathway through a series of changes in AD-related substances.

Outlook

Although current understanding underscores the important role of DNA methylation in the onset of AD, some limitations still need to be addressed. First, most studies have focused on brain tissue from people with advanced AD, and it is unclear whether changes in DNA methylation are already occurring early in this disease. Second, the differences in specific methylation sites and genes observed among different studies may be related to factors such as the sample source, detection method, and data analysis process. Furthermore, DNA methylation patterns are influenced by various internal and external environmental

factors, and these confounding variables need to be better controlled. Román GC *et al*. [91] showed hypermethylation of specific sites in the methylenetetrahydrofolate reductase (*MTHFR*) gene promoter region in prefrontal cortex tissue and peripheral lymphocytes of AD patients after death versus those in healthy controls. Cajavilca CE *et al*. [92] showed that hyperhomocysteinemia is a risk factor for dementia and is expected to be a potential cause and therapeutic target for the most common subtype of dementia. Typically, epigenetics provides a new perspective for studying AD pathogenesis, and DNA methylation is an important entry point. It is necessary to comprehensively clarify the role of DNA methylation in the occurrence and development of AD through larger-scale studies and the integration of multiomics research methods, as well as apply this knowledge in diagnostic and treatment strategies.

In conclusion, this review summarized the role of DNA methylation in AD occurrence, providing a theoretical basis for the early prevention and treatment of this disease.

Availability of Data and Materials

Not applicable.

Author Contributions

BYF, JLZ, YC, JF and KZ conceptualized, wrote, and edited the manuscript. BYF, JQW, YGH, YHH and KZ performed the literature survey, drafted and edited the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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