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Review Article

DNA methylation in the pathology of Alzheimer's disease: From gene to cognition

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Abstract

Alzheimer's disease (AD) is a debilitating disorder that manifests with Aβ plaque deposition, neurofibrillary tangles, and neuronal loss, leading to severe cognitive impairment. Although much effort has been made to decipher the pathogenesis of this disease, the mechanisms causing these detrimental outcomes still remain obscure. In past decades, neuroepigenetics has emerged as an important field that explores how reversible modifications can change gene expression to control behavior and cognitive abilities. Among epigenetic modifications, DNA methylation requires further elucidation for the conflicting observations from AD research and~~has attracted the most attention due to~~ its pivotal role in learning and memory. In this review, we focus on the essential components of DNA methylation, the effects of aberrant methylation on gene expressions in the amyloidogenic pathway and neurochemical processes, as well as memory epigenetics in AD.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by neurofibrillary tangles (NFT), amyloid-beta (Aβ) plaques, and neuronal loss, leading to impaired memory and cognition [1]. Despite decades of research on the wide spectrum of pathologies in AD, we are still no closer to understanding the causes. Although some genes are known to increase the risk of AD, few causative genetic factors have so far been reported. The sporadic nature of AD was demonstrated in a study conducted on monozygotic twins, only one of whom was diagnosed with AD despite both sharing the same genetic code [2]. This finding further substantiates the role of non-genetic components in AD pathophysiology. Considering that manifestations of AD predominantly appear in aged subjects, this complex disease is likely contributed by environmental factors during the course of aging. Epigenetics has been regarded as the genetic response that[3]~~This suggests the involvement of epigenetics, which~~ links environmental influences with altered gene expression and phenotype [3]. Indeed, collective studies in the field of neuroepigenetics have suggested an association between dysregulated epigenetic modifications and AD [4]. Among the epigenetic mechanisms, histone modifications are relatively more established in terms of their role in AD. Treatments based on histone deacetylase inhibition have shown promising progress in drug development [5]. On the other hand, the observations of DNA methylation of disease target genes (see below) in AD is rather contradictory, and hence require an in-depth inspection to further dissect the molecular pathology of AD. In particular, DNA methylation has been extensively investigated regarding its association with aging, memory, cognition, and AD [6-8]. This review briefly discusses the mechanisms of DNA methylation. Next, we discuss the contribution of DNA methylation in AD pathology by outlining features across brain regions. Lastly, we examine how altered DNA methylation in the amyloidogenic pathway and neurochemical processes affect memory.

Mechanism of DNA methylation

DNA methylation refers to the covalent addition of methyl groups to the 5' position of cytosines on DNA to produce 5-methylcytosine (5mC). This process takes place mainly in genomic regions abundant in CpG dinucleotides called CpG islands, which are found in both promotors and gene bodies [4, 9, 10]. DNA methylation in the promoter results in the outward projection of the bulky additional methyl group, leading to spatial hindrance that prevents the binding of RNA polymerase [11]. These structural changes perturb the attachment of transcription factors to the transcription start site ~~(TSS)~~ suppressing gene transcription. In contrast, methylation of CpG in gene bodies is reported to be associated with transcription promotion [1, 11]. In addition to its major roles in regulating gene expression, the role of DNA methylation in directing cell differentiation has also been extensively studied. It was previously believed that the DNA methylation profile remains static after embryonic development [12], but there is growing evidence that demonstrates the DNA methylation pattern in the brain continues to change with age [6]. Studies have also demonstrated that DNA methylation has essential roles in memory [13, 14]. Besides mediating cognitive behavior, DNA methylation is also believed to be an integral component in many neurodegenerative disorders such as Alzheimer's disease [15, 16]. The underlying DNA methylation machinery consists mainly of a class of enzymes known as DNA methyltransferases (DNMTs). These DNMTs generally catalyze the conjugation of methyl groups to cytosines, but different subtypes have different roles in cellular processes. The maintenance methyltransferase DNMT1 mediates the methylation of hemimethylated daughter strands during DNA replication to ensure conservation of the DNA methylation pattern and to maintain DNA methylation already present in quiescent and post-mitotic cells [17]. On the other hand, DNMT3A and 3B methyltransferases are responsible for *de novo* DNA methylation [2], which are directly counteracted by TET1 and GADD45B that mediate DNA demethylation [7, 18]. Together, these molecular players control the dynamic nature of DNA methylation.

Characteristics of regional DNA methylation changes in AD brain

The intricate cytoarchitecture and complex structure of the brain allows the division of labor among different brain regions. In the case of memory, the hippocampus is responsible for memory formation, whereas a range of structures including the basal amygdala and prefrontal cortex are responsible for memory consolidation and storage processes [19]. This implies memory is influenced by complicated processes involving various regions of the brain. However, whether these processes are mediated by DNA methylation remains unclear [20]. Given the aforementioned observations that support DNA methylation as a major mechanism involved in memory, the pathology behind memory loss in AD might also be related to regional dysfunctions in the brain, as DNA methylation changes in AD brain can differ from region to region.

In an attempt to find such patterns in AD, efforts have been made to map out DNA methylation changes across brain regions. The DNA methylation status in the hippocampus is of particular interest due to its established role in cognitive functions. Chouliaras et al. [21] conducted an immunohistochemistry study of DNA methylation in the hippocampus. They quantified the amount of 5mC staining in the CA1, CA3, and dentate gyrus (DG), and further divided the results across neuronal and glial cells. Overall, the hippocampus of AD patients had reduced levels of 5mC compared to the controls [21]. More specifically, there was decreased 5mC in both cell types in CA1 and in glial cells in CA3 [21]. In agreement with Mastroeni et al. [15], Chouliaras et al. also observed the DNA methylation level was negatively correlated to both the amyloid plaque and NFT loads in the hippocampus, which further validates the role of DNA methylation in AD pathology [21]. A later study also reported global DNA hypomethylation accompanied by decreased DNMT1 and DNMT3A~~a~~ expression levels in the hippocampus from postmortem AD samples [16].

The hippocampus is not the only brain region exhibiting DNA methylation changes in AD. Regions that are functionally or structurally connected to the hippocampus, such as the entorhinal cortex, temporal cortex and frontal cortex, were also observed to have altered DNA methylation in AD. Hypomethylation was found in the entorhinal cortex of AD patients together with significantly lower levels of both 5mC and 5-methylcytidine compared to cognitively intact individuals [15]. The entorhinal cortex is spatially close to the hippocampus and functionally connected to it by neuronal projections. It is involved in relaying information between the hippocampus and other cortical and subcortical regions [22]. Moreover, atrophy of the entorhinal cortex is one of the earliest structural changes in AD that precedes atrophy of the hippocampus [23]. Considering its involvement in memory and AD progression, it is not surprising that DNA methylation changes are observed in the entorhinal cortex in AD.

The temporal cortex is a more general region of interest that encompasses both the hippocampus and entorhinal cortex. In a study investigating DNA methylation differences in postmortem brains of a pair of monozygotic twins with discordant AD status, they found significantly decreased expression levels of DNA methylation markers in the anterior temporal cortex of the twin suffering from AD compared to the control twin [2]. These differences were not just in neurons, but also in astrocytes and microglia, which is consistent with the findings in the hippocampus. On the contrary, there have also been reports of DNA hypermethylation in the medial temporal gyrus in AD samples [24]. The reason behind such contradictory results is not entirely clear, but may be due in part to slight differences in the sampled regions. Besides, it remains unclear whether the hypomethylation in the hippocampus and entorhinal cortex extends to the whole temporal cortex.

The frontal cortex is another region of interest, but the findings are less certain. On the one

hand, DNA hypomethylation was observed in the superior frontal gyrus in AD [2]. However, DNA methylation levels were increased in the medial frontal gyrus. The DNA methylation level was positively correlated with AD pathology, as seen from parallel increases in DNA methylation with NFT and Aβ loads [24]. The complexity of the DNA methylation changes in the AD frontal cortex was investigated by a genome-wide methylation analysis. The result revealed that DNA methylation changes occurred in both directions in a gene-specific manner, i.e., hypermethylated genes were mainly related to the regulation of transcription and gene expression, whereas hypomethylated genes were largely related to protein metabolism and membrane transport [25]. Given the functional differences in these genes, it is important to consider the genomic distribution of the DNA methylation changes, aside from the regional features, in order to decipher the effects in AD (**Table 1**).

Alzheimer's disease encompasses a wide spectrum of pathologies and is thus too complex a disease to limit the discussion of altered DNA methylation to any specific disease pathway. Therefore, the following sections will examine the contribution of aberrant DNA methylation in the pathological pathways and neurochemical processes in AD.

Genetic components in AD

Amyloidogenic pathway

The main etiological hypothesis of AD is the amyloidogenic pathway. This pathway involves the cleavage of the amyloid precursor protein (APP) at two sites by β-secretase and γ-secretase, respectively, resulting in the release of Aβ42, the principal deposit observed Aβ plaques in the brains of AD patients (see **Figure 1**). Accumulation of Aβ plaques is a prominent pathological hallmark of AD. Aberrant processing of the APP protein is directly linked to the development of AD. Early studies observed increased APP expression in familial Alzheimer's disease and Down's syndrome patients, which are both

caused by mutations of their genes on chromosome 21 [26, 27]. Coincidently, Down's syndrome patients are predisposed to AD and tend to develop AD-like dementia in middle age [28]. To establish age as a factor that influences the pathology of AD from an epigenetic perspective, Tohgi et al. [29] compared the degree of methylation in the promoter regions of APP in the cerebral cortex from aged (>70 years) and normal (<70 years) AD samples. They found significant hypomethylation at APP promoter regions in the aged samples. In line with this finding, another study assessed AD-related gene methylation in peripheral blood leucocytes of diagnosed AD patients, which revealed decreased DNA methylation at the APP promoter regions accompanied by upregulated APP transcripts [30]. West and colleagues [31] detected hypomethylation in the CpG-rich promotor region of the APP gene in AD temporal cortex. A later study found increased methylation within the exonic region of APP [32]. Although the alterations may be in opposition, both hypomethylation and hypermethylation collectively pointed to increased APP expressions in AD. These results suggest that CpG islands upstream of APP gene could be manipulated as a possible therapeutic strategy. Nonetheless, whether DNA methylation of the promoter region of the APP gene accurately reflects disease state remains controversial, as some recent studies found no significant differences in the methylation state of normal control and AD patient samples [33, 34]. Interestingly, the Aβ deposit itself was demonstrated to have an influence on epigenetic regulation. Murine cerebral endothelial cells exposed to Aβ peptides showed a significant reduction of the global DNA methylation level [35]. Such an observation was also reported in an *in vivo* study. The McGill-Thy1-APP mouse model, a commonly used AD mouse model, displays early Aβ deposition in the subiculum that spreads into the hippocampus and cortex [36]. Screening of global DNA methylation level in different brain regions in this mouse model corresponded with the spatial pattern of Aβ pathology [37]. Immunofluorescence co-labeling was used to compare 5mC levels in regions affected by Aβ, which further confirmed a severe reduction of neurons compared to the total cell population, implying that Aβ primarily acts on neurons to induce hypomethylation [37]. Taken

together, the above findings provide growing evidence to support the involvement of Aβ in reducing global DNA methylation in the initial disease stage, and Aβ accumulation in later stages then further promotes neuronal DNA demethylation.

Besides upregulation of the APP gene, *BACE1*, the major form of β-secretase expressed in the brain, was also shown to have a direct effect on Aβ deposition in the brain. Cortical neurons cultured in BACE1-deficient medium successfully abolished Aβ secretion. This finding was further validated by an *in vivo* experiment, in which knockout of ~~*BACE1*~~ *Bace1* in a mouse model overexpressing human APP significantly reduced Aβ generation [38]. A similar observation was reported in monkeys, in which infantile monkeys exposed to lead metal, a risk factor for AD, showed increased expression of *BACE1* mRNA in the cortex with age [39]. This was likely mediated by DNA hypomethylation, as the lead-exposed animals also exhibited a 20% decrease in DNMT1 activity [39]. A recent genome-wide screening study of enhancer DNA methylation in the prefrontal cortex of AD patients revealed a hypomethylated ~~*BACE1*~~ *BACE1* enhancer, which was associated with increased ~~*BACE1*~~ *BACE1* expression and amyloid plaque deposition [40]. In line with these results, a correlation study demonstrated hypomethylation in the ~~*bace1*~~ *BACE1* promotor, with the methylation level of one CpG negatively correlated to the Aβ load, whereas the methylation level of another CpG was correlated to the rate of cognitive decline in AD patients [37]. Such findings support the reduction of ~~*bace1*~~ *BACE1* gene methylation in AD may possibly contribute to the accumulation of Aβ and severity of disease progression. Presenilin 1 (~~*psen1*~~*PSEN1*), another gene of interest in AD pathology, encodes one of the core proteins of γ-secretase that is responsible for the final cleavage of Aβ from APP. As both enzymes work in concert, it would be of interest to investigate whether *psen1* shares the same methylation changes as ~~*bace1*~~*BACE1*. Indeed, candidate gene methylation analysis revealed *PSEN1* gene exhibits the strongest disease-specific effect in late-onset AD brains among all candidates, albeit the results should be interpreted with caution due

to high interindividual variance [34]. Given their integral roles in the resulting Aβ pathology, these genes have attracted plenty of attention in transgenic AD rodent studies and disease profiling studies. The AD-associated changes in DNA methylation can also be indicated by anomalies in the metabolite levels of AD patients, as demonstrated by increases in plasma levels of homocysteine (HCY) and simultaneous decreases in serum levels of folate and vitamin B12 [41-43]. Recent studies on one-carbon metabolism indicated a more causal relationship between DNA methylation and the expression of secretases leading to the production of the neurotoxic Aβ42 and AD neuropathology [44]. One-carbon metabolism consists of the folate and methionine cycles. The former process requires the intake of folate for the transfer of a methyl group in the synthesis of methionine in the latter cycle leading to the production of S-adenosylmethionine (SAM) (see **Figure 2**), which is an essential component for sustaining normal DNA methylation by acting as the primary methyl donor. The downstream molecules of SAM, S-adenosylhomocysteine (SAH) and HCY, are potentially cytotoxic substances and are known DNMT inhibitors that have a substantial effect on inducing hypomethylation [45-48]. Under normal conditions, SAM will donate a methyl group to the substrate before transforming to SAH, which undergoes hydrolysis to give HCY [49], and SAM can then be regenerated from HCY via the formation of methionine by transmethylation, a metabolic cycle that requires constant supply of folate and vitamin B12. In a folate and vitamin B12-deficient environment, the imbalanced one-carbon metabolism leads to disturbed transformation of HCY and reduced SAM production, which in turn affects downstream DNA methylation and increases amyloidogenesis possibly through the upregulation of ~~*BACE1*~~ *Bace1* and ~~*PSEN1*~~ *Psen1* transcripts [50, 51]. Such dietary deficiency can significantly exacerbate Aβ pathology and cognitive deficits in TgCRND8 mice, a mouse model that contains multiple familial AD mutations and is characterized by early disease onset [51, 52]. Several components in one-carbon metabolism, such as methylenetetrahydrofolate reductase (MTHFR) and folic acid, have been shown to be associated with late-onset AD. A mutation in the MTHFR gene and low dietary intake of

vitamin B9 and B12 may delay the clearance of HCY and eventually results in elevated levels of HCY in the plasma [53]. The above dietary deficiency was also found to lower the activity of DNMTs, while at the same time enhancing the activity of DNA demethylase, which may exert a large-scale effect on genomic regulation [54]. Clinically, a reduced level of SAM was detected in the cerebrospinal fluid of AD patients [55], further strengthening the significance of the methionine cycle on AD pathology. Ultimately, all these factors impede DNA methylation, supporting the above observations of decreased DNA methylation in AD brains. In line with these observations, promoting SAM levels by exogenous administration was demonstrated to restore the DNA methylation level of BACE1 and cognitive deficits *in vivo*, as well as normalize the transcript levels of both BACE1 and PSEN1 *in vitro* [37, 50]. Such an intervention is believed to counteract the adverse effects of Aβ on global DNA methylation status by replenishing the supply of substrates in the methionine cycle and restoring DNA methylase/demethylase activity [54].

Apart from inducing widespread DNA hypomethylation, it was reported that Aβ deposition could also simultaneously cause hypermethylation with the subsequent downregulation of specific genes, which in turn favors the accumulation of amyloid plaques. Some prominent examples are neuronal sortilin-related receptor (SORL1) and neprilysin (NEP). ~~Neprilysin~~NEP is an Aβ degrading protein (thought to be a major Aβ degrading protease *in vivo*) that enhances the clearance of amyloid plaque deposits via enzymatic cleavage, whereas SORL1 plays an essential role in directing the APP holoprotein to recycling pathways, thereby inhibiting the production of neurotoxic Aβ [56-58]. DNA methylation of *SORL1*, along with several other gene loci (*ABCA7, HLA-DRB5, SLC24A4 and BIN1*), was found to be associated with AD pathology [59], although whether Aβ has a causative role in the methylation status of these genes remains unknown. Among these genes, the expression of *SORL1* in AD has been the most characterized, with levels downregulated in sporadic AD patients [60, 61], whereas overexpression of SORL1 significantly reduced Aβ

production *in vitro* [62]. In one *in vitro* study, they showed that Aβ indeed reduced the global DNA methylation as aforementioned, but they also found that Aβ could induce hypermethylation at ~~*NEP*~~ *Nep* promoter regions [35]. However, another study reported no differences in *NEP* promoter methylation in post-mortem AD brains [63]. These conflicting results could possibly be due to the heterogeneity of the research methods (High-performance liquid chromatography versus pyrosequencing) or sampling (cultured cell versus human sample). Modulation of NEP as a therapeutic target may be a promising therapeutic strategy, as an *in vivo* study demonstrated the overexpression of NEP prior to the onset of Aβ pathology partially restored memory deficits along with reducing amyloid plaques in young transgenic AD mice [64]. Besides facilitating endosomal recycling of APP, the clearance of toxic Aβ produced in the amyloidogenic pathway would be equally important as a therapeutic strategy against AD. Considering transcriptions of the above target genes were demonstrated to be governed by DNA methylation, more research focusing on the methylation profile of genes responsible for APP production, processing, and Aβ clearance are warranted.

In view of the above findings, it is plausible that the disruption of DNA methylation at several checkpoints in the amyloidogenic pathway, possibly caused by an imbalance in one-carbon metabolism, may contribute to the pathophysiology of AD. In addition to the amyloid-related pathology, deficits in plasticity and memory-related genes are also equally responsible for the disease manifestations in AD. Impairments in neurochemical processes downstream of the Aβ-related pathology, such as neurogenesis [65], cell survival [66], and synaptic plasticity [67], have been consistently reported in AD (See **Figure 3**). In the following sections, we will highlight the research on cellular components involved in learning and memory and discuss their altered methylation status in the context of AD.

Neurochemical processes

Genetic predisposition and epigenetic mechanisms have been consistently reported to play critical roles in AD pathogenesis [68-70]. These observations were further substantiated in the study by Mastroeni et al. [15], which found reduced 5mC staining and decreased expression of several DNA methylation factors, such as DNMT1 and MeCP1/MBD2 complex, in the entorhinal cortex of AD post-mortem brains. Given the role of epigenetics in global genomic regulation, the consequences of these epigenetic alterations in AD will be rather diverse. Previous gene ontology studies reported hypermethylation in genes associated with the cell cycle and Wnt signaling, both of which are essential in neurophysiological processes that mediate cognitive functions, including neurogenesis and synaptic plasticity [71-73]. Impaired neurogenesis was consistently reported in transgenic AD mouse models, with the exceptions of a few mouse models harboring Thy1-APP_{SWE} and PDGF-$APP_{SWE/Ind}$ mutations [74]. DNA methylation was found to be essential in mediating neuronal proliferation, differentiation, and survival, as exposure to DNMT inhibitor 5-aza-cytidine was able to disrupt neural stem cell migration and differentiation [75]. Functions of the DNA methylation machinery were found to be specific to different neurogenesis stages. For example, neuronal survival was demonstrated to be dependent on DNMT1, as knockout of *Dnmt1* in neural stem/precursor cells resulted in normal proliferation and differentiation, but failure to reach maturation [76]. Genome-wide analysis revealed DNMT3A~~a~~ promoted transcription of neurogenic genes by mediating methylation on their corresponding non-proximal promoters, which was necessary for neuronal differentiation [77]. Growing evidence points to reduced efficacy of DNA methylation in AD, as reflected by downregulated DNMTs and reduced levels of 5mC in the entorhinal cortex and hippocampus of AD brains [15, 16, 21]. Therefore, altered DNA methylation may be a potential mediator of impaired neurogenesis in AD. Moreover, Dickey et al. [78] demonstrated a partially causative role of Aβ deposits on synaptic dysfunction in AD. They observed a consistent downregulation of genes involved in long-term potentiation (LTP), such as early growth response protein 1 (*Egr1*) and activity-regulated cytoskeleton-

associated protein (*Arc*), in amyloid-containing brain regions of APPxPS1 transgenic mouse. Although there was relatively intact synapse structure, as indicated by the normal expressions of genes responsible for presynaptic vesicle transport, this study still provided evidence to support impaired synaptic plasticity in AD. The *Egr1* and *Arc* genes are categorized as immediate early genes (IEGs), which have a wide spectrum of functions in the neural circuit due to their rapid and transient nature. Specifically, these two genes are needed for synaptic plasticity, a process that precedes memory formation [79]. The consequence of *Egr1* downregulation is thought to be manyfold, as it is an essential transcription factor. Deletion of *Egr1* was reported to induce long-term memory and LTP deficits [80]. Several studies showed that *Egr1* has the ability to coordinate neurochemical processes through regulating expressions of genes involved in synaptic architecture, neurotransmitter release, and protein trafficking [81, 82]. Recently, it was found that *Egr1* works in concert with TET1 to facilitate neuronal activity-induced demethylation and subsequent downstream gene transcription [83], which further demonstrates the ability of *Egr1* to orchestrate gene expression. Although this finding at first appears to be contradictory to the DNA hypomethylation observed in AD, several preclinical and clinical models also reported controversial results regarding the expression level of *Egr1*. Upregulation and gradual reduction of *Egr1* transcript levels were detected in post-mortem brains of patients in early and late stages of AD, respectively [84]. Whether the upregulation of *Egr1* transcripts promotes TET1-mediated demethylation to pave the way for global hypomethylation in AD requires further exploration. As an IEG and an essential protein in cognitive functions, *Arc* facilitates the association between environmental cues and spatial learning [85, 86]. Generally, *Arc* is used as a marker for locating synaptic activation given its rapid expression upon neuronal activity [79, 87]. Despite its well-documented role in the induction of early-phase LTP via regulating AMPA receptor endocytosis and in mediating synaptic plasticity, the absence of *Arc* abolished the transition from early-phase to late-phase LTP [88, 89]. As the initiation and maintenance of late-phase LTP requires gene

transcription [90], this implies that *Arc* may be involved in transcription regulation. Indeed, RNA-sequencing combined with gene ontology revealed *Arc* has an inhibitory effect on Aβ-related processes and NFT [91]. Besides, *Arc* is also thought to have significant effects on the expression of AD susceptibility genes. The absence of *Arc* expression was associated with the downregulation of genes highly implicated in memory such as brain-derived neurotrophic factor (*Bdnf*) and calcium/calmodulin dependent protein kinase IV (*Camkiv*); genes associated with tau protein pathology such as Neuronal PAS domain protein 4 (*Npas4*) and dual specificity tyrosine-phosphorylation regulated kinase 2 (*Dyrk2*); and genes alleviating Aβ pathology such as Integral membrane protein 2B (*Itm2b*~~ITM2B~~) and LDL receptor related protein 1 (~~*LRP1*~~*Lrp1*) [91]. These findings further support the essential roles of Arc, as well as consequences of a dysregulated genomic network in AD. In spite of its apparent importance, studies of *Arc* methylation in AD are surprisingly scarce. Clinically, hippocampal CA1 neurons bearing NFT have more than a three-fold decrease in *Arc* expression in AD [92]. Interestingly, another study reported upregulated *Arc* expression in the medial frontal cortex in post-mortem AD patients [93], which indicates possible temporal differences in *Arc* expressions in AD. However, what mediates such changes is unclear, and it would be of interest to determine if DNA methylation plays a role. The methylation status of *Arc* in cognition has been studied in relatively more detail. Regulation of *Arc* by DNA methylation was reported in aged mice, in which aberrant methylation of *Arc* in hippocampal subregions led to decreased *Arc* mRNA and following depressive-like behavior, with corresponding deficits in spatial memory in behavioral tasks [94]. The presence of *Arc* appears to be indispensable in mediating memory functions, and knockdown of *Arc* in the lateral amygdala, hippocampus, or anterior cingulate cortex impaired long-term memory [95-97]. The dynamics of *Arc* expression was shown to be regulated by time-dependent DNA methylation and demethylation orchestrated by DNMT3A and Gadd45γ, respectively, and the latter was required for long-term memory consolidation [98]. In a study by Wu et al. [93] using cultured neurons and transgenic APP_{SWE};

PS1Δ E9 mice that lack the expression of *Arc*, they confirmed that *Arc* facilitated Aβ plaque formation and deposition via binding to PS1 *in vitro* and *in vivo*. In line with the above result, *Arc* was also found to be upregulated in close proximity to the amyloid plaque, further reinforcing its association with the pathophysiology of AD [99]. However, some *in vivo* research employing transgenic mouse lines have reported controversial findings on *Arc*, with upregulation of *Arc* in some studies [93, 100] and reduced *Arc* transcripts in other studies [101]. These inconsistent observations on the role of *Arc* in AD-associated pathology could be attributed to differences in methodologies employed in individual studies, such as biochemical assays, strains of transgenic animals, and the disease stage represented by different animal models. Nonetheless, although the tight association between these two IEGs and DNA methylation has consistently been shown to play a critical role in memory, more investigations are warranted to examine the disease-modifying nature of these IEGs in AD.

Besides these IEGs, BDNF has also recently gained attention as a potential therapeutic target in AD. As a member of the neurotrophin family, BDNF binds to tropomyosin-related kinase receptor type B (TrkB) to promote neuronal survival and mediate several essential functions including dendritic spine modulation, LTP, and synaptic plasticity. The BDNF protein is highly expressed in the hippocampus and cortex following excitatory signals, where it promotes survival of neurons, such as cholinergic neurons, which is highly implicated in AD [102]. The importance of BDNF-TrkB has been well documented. Chronic exogenous BDNF delivery in the hippocampus significantly promoted neurogenesis in the granule cell layer [103], whereas mutant mouse expressing a truncated form of *Bdnf* mRNA exhibited deficits in the differentiation of new neurons in the subgranular zone [104]. In AD, gradual synapse loss affects brain areas essential to memory such as the entorhinal cortex and hippocampus [105, 106], and deficits in BDNF levels have also been reported in these regions [107-109]. Replenishing BDNF in the entorhinal cortex showed promising results

in restoring synaptic markers in AD transgenic animal models, indicating a potential reversal of synapse loss induced by Aβ and tau-related pathologies [110, 111]. However, such a therapeutic effect is thought to act independently of APP and tau protein phosphorylation, as localized BDNF delivery did not affect amyloid plaque deposition or tau hyperphosphorylation level [110, 111]. This indicates that BDNF may be a viable therapeutic target of AD. Moreover, emerging evidence suggests that the diverse functions of BDNF and its modulation through epigenetic modifications make it an integral candidate for research in various neurological and psychiatric disorders [112]. Specifically, the temporal-spatial regulation of ~~*BDNF*~~ *Bdnf* relies on the differential methylation of various promoters of *Bdnf* exons in response to distinct stimuli [113]. Such precise *Bdnf* exon-specific transcription is orchestrated by a series of transcription factors, including cyclic-AMP response element binding protein (CREB), NPAS4, and methyl-CpG binding protein 2 (MeCP2) [114]. Notably, MeCP2 was demonstrated to act as a constraint by repressing *Bdnf* exon IV expression via binding at the promoter regions. Such constraint can be relieved by neuronal activity-dependent phosphorylation at Ser421 and 424 leading to the dissociation of MeCP2 from the promoter [115, 116]. Recent studies on epigenetic modifications in AD animal models with Aβ and tau-related pathologies showed upregulated MeCP2 levels in the hippocampus [117, 118]. Although an increased level of phosphorylated MeCP2 (pMeCP2) was simultaneously observed in these AD animals, the phosphorylated site was reported to be at Ser80, whereas pSer421 remained unchanged [118]. Hence, it is possible that Aβ and tau-related pathology work in concert to suppress *Bdnf* expression, which disrupts normal synaptic functions, resulting in synaptic impairment and neuronal loss. In line with the above results, multiple studies on the methylation of ~~*BDNF*~~ *BDNF* gene in human AD samples showed the promoter of ~~*BDNF*~~ *BDNF* was hypermethylated in the hippocampus, temporal and frontal cortex, and was accompanied by reductions in ~~*BDNF*~~ *BDNF* mRNA and protein levels in these regions [102, 119-121]. Notably, the increase in methylation was positively correlated to the duration of illness, but negatively correlated to recall ability [120].

The possibility of using the methylation status of peripheral blood BDNF as a marker to evaluate the risk of AD was also assessed, as AD patients exhibited higher levels of CpG methylation in the promoter regions of BDNF [122]. On the other hand, the sustainability of activity-dependent *Bdnf* transcription appears to require the binding of NPAS4, as disruption of NPAS4 function reduced *Bdnf* promoter IV transcription [123]. Apart from regulating *Bdnf* transcription, NPAS4 was demonstrated to control the balance of excitatory and inhibitory synapses [123]. Interestingly, APP overexpression, but not Aβ plaque deposition, induced hyperexcitability characterized by the presence of sharp wave discharges observed in the electroencephalogram along with elevated GABAergic innervation and decreased glutamatergic innervation, thus altering the balance between excitatory and inhibitory neurotransmission [124]. These outcomes were thought to be mediated by NPAS4, as genetically silencing ~~*NPAS4*~~ *Npas4* downregulated GABAA receptor alpha 1, a phenotype observed in APP-deficient cultured neurons [125]. Reduced expression of ~~*NPAS4*~~ *Npas4* mRNA and attenuated LTP were detected in McGill Thy1-APP transgenic AD mouse model [126], suggesting possible NPAS4-mediated transcription deficits in AD. Intriguingly, NPAS4 was not only found to be involved in the pathology downstream of APP generation, but also played a critical role in clearance of endogenous tau protein. Recently, a tau clearance pathway was identified that was mediated by autophagy and facilitated by the overexpression of ~~NPAS4~~NPAS4, which resulted in an overall decrease in tau and phosphorylated tau protein levels [127]. Despite NPAS4 being a potentially promising therapeutic target in AD, there is still insufficient information on its regulation by DNA methylation. There has been only one preclinical study that showed modulation of *Npas4* transcription by DNA methylation [128]. Although this study was conducted in a stress animal model, it still provides evidence from an epigenetic perspective for NPAS4 as a possible target. Another molecule involved in tau protein pathology is glycogen synthase kinase 3β (GSK3β). The hyperphosphorylation of tau protein by GSK3β facilitates the formation of tangle-like filaments that constitute NFT [129]. A

further study identified hypomethylation of *GSK3β* in an *in vitro* experiment mimicking AD-related pathology and in post-mortem AD samples [130]. Intriguingly, only patients in the initial disease stage exhibited GSK3β upregulation [130]. A memory-enhancing gene, reelin (*RELN*), has also been highly implicated in AD. It was shown to bind apolipoprotein E receptor 2 (ApoER2) or very-low-density liporeceptor to facilitate migration of neurons and synaptic transmission [131, 132]. The expression of ~~*reelin*~~ *Reln* was found to be regulated by DNA methylation, as upregulated ~~*reelin*~~ *Reln* mRNA expression was accompanied by a decreased methylated promoter region in contextual fear conditioning [14]. The methylation status of ~~*reelin*~~ *Reln* could also be modulated by exogenous supplements of components involved in one-carbon metabolism. Chronic administration of high-dose L-methionine induced hypermethylation in the promoter region of reelin and produced a disease state that mimicked schizophrenia [133]. Collectively, transcriptional activation of ~~*reelin*~~ *Reln* was beneficial to cognitive ability and neurotransmission. In AD, downregulation of ~~*reelin*~~ *Reln* was associated with increased tau phosphorylation and accelerated Aβ plaque deposition [134, 135]. Surprisingly, recent studies demonstrated a possible compensatory mechanism that upregulated ~~*reelin*~~ *Reln* expression after Aβ-induced disruption of ~~reelin~~ RELN conformation impaired its activity and compromised ~~reelin~~RELN-ApoER2 signaling [136, 137]. However, such a compensatory increase in ~~reelin~~ RELN level did not appear to be mediated by DNA methylation, as the promoter methylation remained unchanged in both Aβ-treated neuroblastoma cell line and frontal cortical genomic DNA extracts from AD patients [136]. In view of this finding, it is likely that other epigenetic mechanisms, for example histone modifications, contribute to the observed elevation of RELN, as histone acetylation was shown to be involved in synaptic plasticity and memory [138]. Besides its interaction with Aβ, ~~*reelin*~~ *RELN* was also found to be negatively associated with the progression of the NFT pathology [139]. These results support the increase of ~~reelin~~ RELN activity as a potential therapeutic strategy against AD. Indeed, ~~reelin~~ RELN was able to rescue Aβ-induced attenuation of LTP and NMDA receptor current through downstream

signaling cascade activation of ApoER2 [140]. Given that APP/Aβ alters global DNA methylation, more investigations identifying gene expressions that are affected by AD pathology will prove to be valuable in understanding AD pathogenesis.

On the other hand, because of its capacity for sustained alterations and widespread control over gene expressions, DNA methylation is regarded as a critical mechanism that induces and maintains changes in synaptic plasticity required for memory formation by repressing genes that suppress memory [8]. As DNMT activity tightly regulates DNA methylation, several studies have examined DNMT in association with memory formation. It has long been established that gene transcription is essential to induce synaptic plasticity, a process that builds the foundations of memory [90]. Indeed, DNA methylation was shown to play a major role in this important neurophysiological process. Levenson et al. [141] demonstrated DNMT activity was necessary for inducing long-term potentiation in the hippocampus. Miller and Sweatt [30] investigated changes in DNMT expression levels in rats following contextual fear conditioning by directly examining the genes involved in memory formation associated with aversive stimuli and environmental cues. They found increased mRNA levels of ~~*DNMT3A*~~ *Dnmt3a* and *3b*~~3B~~ (DNMTs that are responsible for de novo methylation) in the hippocampal CA1 region shortly after the training session [14]. The functional contribution of DNMTs to memory formation was further substantiated in animal studies. Immediately after conditioning, DNMT activity in the brain was suppressed by DNMT inhibitors, resulting in less freezing behavior, which is indicative of disrupted memory consolidation [13, 14]. This revealed a tight link between the DNA methylation machinery and memory. Other studies identified several genes that exerted significant effects on mediating memory formation. Based on their activity during the course of memory consolidation, they were categorized into memory-enhancing or memory-suppressing genes. As memory loss is a key symptom of AD, it is possible that these memory defects are due to abnormalities in the DNA methylation machinery in AD brain. A

study using both nuclear and cytoplasmic staining to assess the level of DNA methylation in the entorhinal cortex of AD samples revealed markedly reduced immunoreactivity of DNA methylation markers in AD [15], which was accompanied by decreased immunoreactivity of not only DNMT1, but also components of the DNA methylation stabilizing complex (including MTA2, HDAC1, HDAC2, p66α, RbAp48, and MBD2/3) [15]. Moreover, the immunoreactivity of DNA methylation markers was inversely related to that of late-stage NFT markers, which suggests the decreased DNA methylation in AD may contribute to the dysregulation of these pathological markers [15]. Altogether, this result confirms a decreased DNMT level alongside DNA methylation level in AD brain, further hinting at a relationship between reduced DNA methylation and neurodegeneration. Such alterations may lead to a disruption in the balance between memory-enhancing or memory-suppressing genes, and disruption of essential memory formation processes.

These current perspectives on the neurobiology of memory have led to the hypothetical classification of memory-enhancing and memory-suppressing genes that are regulated by DNA methylation to facilitate memory formation [8, 14]. Memory-suppressing genes are assumed to be transcriptionally repressed to facilitate memory formation [8]. Calcineurin (CaALNN), a calcium/calmodulin-dependent phosphatase, and its downstream molecule, protein phosphatase 1 (PP1) were first studied using genetic inhibition methods, which established them to function as memory constraints. These constraints were able to be reversed using a conditional knock-out animal model [142, 143]. Such observations suggested a dynamic and reversible regulatory mechanism was involved in governing the expression of the aforementioned genes to facilitate memory formation. Recent neuroepigenetic studies that extensively characterized the expressions of these genes in different stages of memory support this notion, as *PpP1* was significantly downregulated with increased DNA methylation after contextual fear conditioning [14]. A study targeting *CaalnN* in the anterior cingulate cortex in rats revealed sustained hypermethylation at the promoter regions for 30

days after receiving contextual fear conditioning [13]. The persistent downregulation of *CalnaN* mRNA expression was thought to promote remote memory maintenance, as infusion of DNMT inhibitor at the memory maintenance phase disrupted retrieval of fear memory [13]. Indeed, this finding is in agreement with previous report demonstrating overexpressed *CalnaN* could impair the transition from short-term to long-term memory [144]. As a key enzyme in modulating the intracellular calcium level, CALNaN also serves as a master regulator in several cellular processes that resemble pathological hallmarks of AD, such as Bcl2-associated death protein (BAD)-dependent apoptosis [145], PP1-dependent long-term depression (LTD) [146], and GSK3β-mediated tau phosphorylation [147], through dephosphorylation of its downstream effectors. Given that calcium homeostasis is impaired due to Aβ-related pathology and mitochondrial dysfunction in AD [148], it is plausible to suggest that dysregulated CALNaN signaling plays a critical role in AD pathology. Indeed, administration of CALNaN inhibitor FK506 restored Aβ-induced evoked LTP and dendritic spine deficits [149]. Treatment with FK506 *in vivo* partially normalized neurite structural anomalies indicative of elevated calcium and neuronal stress [150]. Another study employing Tg2576 mouse model, which exhibits Aβ overexpression and hippocampal-dependent memory deficits, used FK506 treatment to restore memory deficits due to fear conditioning paradigm and also normalized pCREB levels, which further validates the therapeutic targeting of CALNaN [151]. Collectively, these results confirm the role of CALNaN as a mediator of Aβ-related pathology. Moreover, the interaction between CALNaN and tau protein has been recently investigated. Although it is one of the phosphatases that can dephosphorylate tau protein, a post-mortem study surprisingly revealed a positive correlation between CALNaN activity and the level of tau phosphorylation [152]. This finding is in line with the hypothesis that GSK3β acts as a downstream mediator of tau protein hyperphosphorylation [147, 153]. However, the contribution of CALNaN in hyperphosphorylated tau still remains controversial, as there is evidence demonstrating that reduced CALNaN activity may be responsible for

hyperphosphorylation of tau protein [154]. Clinically, AD patients were shown to exhibit elevated nuclear levels of CALNaN that was directly correlated with the severity of the dementia progression [155]. The downstream substrate of CALNaN, phosphatase PP1, was shown to have significant involvement in cognitive processes, facilitating dephosphorylation and subsequent inactivation of CREB [156]. Besides its nuclear substrates, PP1 could also dephosphorylate NMDA and AMPA receptors through physically associating with these glutamate receptors, contributing to attenuated synaptic transmission [157, 158]. Interestingly, an *in vitro* study showed cells incubated with Aβ peptide had a dose-dependent inhibitory effect on PP1 activity that was exacerbated by Aβ aggregation [159]. The mRNA level of *PpP1* was also found to be significantly reduced in NFT-bearing neurons in AD patients [92]. As a phosphatase that can dephosphorylate Tau protein, reduced PP1 expression could contribute to Tau hyperphosphorylation and subsequent NFT formation [160]. Although DNA methylation appears to be an effective regulatory mechanism for *CalnaN* and *PpP1* in learning and memory, there is a lack of data that directly shows a link between global DNA hypomethylation and altered phosphatase expression in AD. Emerging evidence supports that modulation of DNA methylation may restore age-dependent cognitive deficits and alleviate AD progression. Exogenous S-adenosylmethionine supplement and *Dnmt3a2* overexpression have shown promising effects in counteracting Aβ-induced cognitive deficits and age-related impairment, respectively [37, 161]. Whether changes in the expression and activity of these memory-suppressing enzymes are mediated via DNA methylation has not been clarified. Nonetheless, considering the resemblance of the outcomes produced by dysregulated phosphatase and the observations in AD models and AD pathogenesis, further studies detailing the interplay between these genes and AD will be required to develop therapeutic strategies for AD pathology.

Conclusion

In this review, we have presented several lines of evidence elucidating the role of aberrant DNA methylation in activating disease genes contributing to Aβ and tau protein-related pathologies in AD, as well as in disrupting genes encoding components essential to normal brain physiology. As a complex and multifactorial disease, AD has been demonstrated to manifest with heterogeneous pathologies. The complexity of AD etiology lies in the contributions from both genetic and environmental factors. Extensive research has been conducted to clarify the underlying mechanisms of AD, but the conflicting results and unsuccessful clinical trials of pharmaceutical interventions has meant we are no closer to finding an effective treatment. Clinically, interindividual variations exist in post-mortem AD samples. Although DNA methylation in post-mortem samples was shown to be stable for up to 72 h [162], different rates of sample degradation during the interval between the patient's death and sample collection still remains a concern. Furthermore, the use of transgenic animals to mimic AD pathogenesis, despite being well established, still do not replicate all AD pathologies, let alone an epigenetic profile resembling that of AD patients. These limitations may, in part, contribute to the inconsistencies observed in preclinical and clinical findings. DNA methylation is a growing field that provides a new perspective on the physiological and behavioral changes caused by this neurodegenerative disorder. Given that APP/Aβ is known to modulate the DNA methylation status and subsequent neurochemical processing of certain genes, restoring the defective one-carbon metabolism or using pharmaceutical interventions that suppress selective pathogenic genes might be a promising strategy against AD. However, there are still huge research gaps regarding the DNA methylation status of confirmed risk gene loci [163]. Future research focused on elucidating the epigenetic status of these candidate genes will likely provide more information on disease development and will play a critical role in screening for therapeutic options.

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Conflicts of interest

All authors declare no conflicts of interest

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Legend:

Figure 1. Altered DNA methylation in the various checkpoints of the amyloidogenic pathway. APP is a transmembrane glycoprotein that undergoes two different pathways to produce either a non-toxic cleaved P3 protein (non-amyloidogenic pathway) or a toxic Aβ oligomer that leads to the deposition of Aβ plaques (amyloidogenic pathway). The different components of the amyloidogenic pathway with those shown to be modulated by DNA methylation highlighted in red. In the amyloidogenic pathway, APP is first cleaved by β-secretase encoded by *BACE1* to produce sAPPβ and CTFβ. The CTFβ undergoes a second cleavage by γ-secretase encoded by PSEN1 to generate Aβ. The resulting Aβ undergoes either NEP-mediated cleavage or aggregation to form Aβ plaques. The genes

transcribing these essential checkpoint molecules have been demonstrated to exhibit hypomethylation at the promoter regions in AD animal models or patients. On the other hand, the non-amyloidogenic pathway prevents the accumulation of toxic Aβ by α-secretase cleavage of APP to produce non-toxic P3. The inability to effectively redistribute APP is considered one of the factors of Aβ-related pathology. Another mechanism that avoids over-processing APP is via SORL1-mediated endosomal recycling and degradation of APP. This protective process is thought to be impaired in AD patients, as downregulation of SORL1 is observed in clinical cases.

APP, amyloid precursor protein; sAPPα, soluble α-APP fragments; sAPPβ, soluble β-APP fragments; CTFα, C-terminal α fragment; CTFβ, C-terminal β fragment; NEP, neprilysin; SORL1, sortilin-related receptor 1; AICD, APP intracellular domain.

Figure 2. One-carbon metabolism components. One-carbon metabolism includes the folate cycle and methionine cycle, which integrates folate intake with components required for DNA methylation. Folate and vitamin B-deficiency are often observed in AD patients. Another altered component in the one-carbon metabolism is the hypermethylation or mutation of MTHFR, which has been implicated in homocystinuria. Impaired function of MTHFR may contribute to inefficient methyl group transfer to homocysteine, affecting production of methionine and S-adenosylmethionine.

MTHFR, methylenetetrahydrofolate reductase

Figure 3. Proposed model of synaptic dysfunction in AD. Increased CALaN expression and activity were observed in transgenic AD animal models. Impaired calcium homeostasis is considered to be one of the factors for elevated CALaN activity in AD. Given the wide variety of its downstream substrates, CALaN is thought to mediate tau protein hyperphosphorylation, PP1-mediated dephosphorylation of AMPAR and NMDAR, and BAD-dependent apoptosis and subsequent neuronal loss. PP1 associates with and

dephosphorylates both AMPAR and NMDAR, decreasing synaptic transmission. Dephosphorylation of subunits in NMDAR is also associated with endocytosis of this receptor, thereby attenuating synaptic strength. The endocytosis of NMDAR may be rescued by reelin, which binds to ApoER2 to activate a downstream signaling cascade via SFK to enhance NMDAR subunit phosphorylation. Furthermore, both nuclear CALaN and PP1 were shown to dephosphorylate, and hence inactivate CREB, which may result in transcriptional repression of *Bdnf*. This may further exacerbate the synaptic dysfunction in AD. GSK3β is activated upon dephosphorylation of CALaN, which facilitates increased tau protein phosphorylation and contributes to microtubule depolymerization.

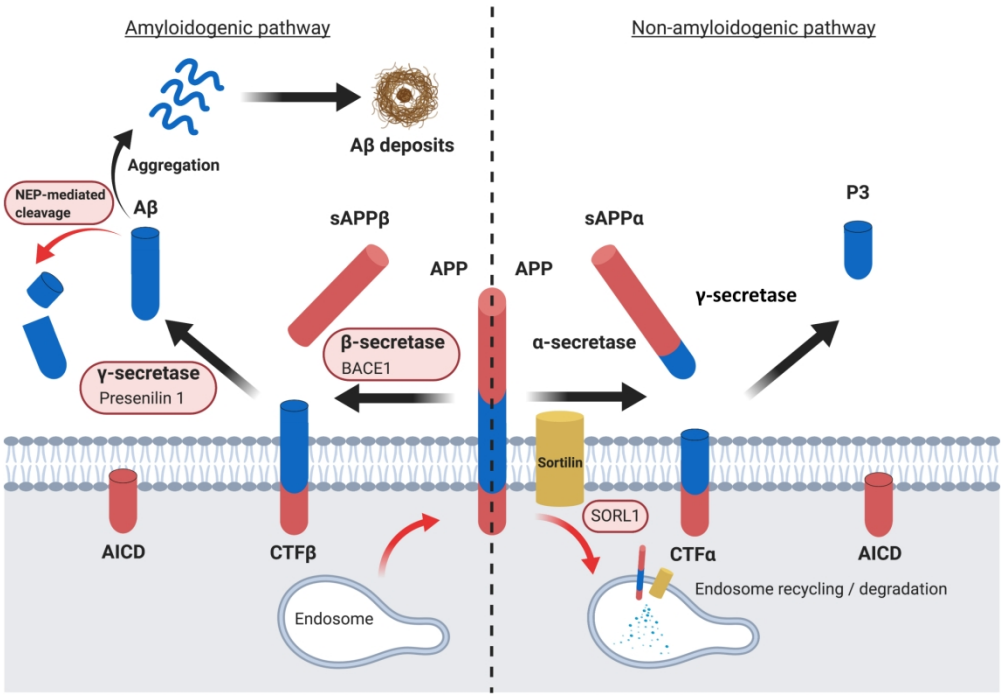
CALaN, calcineurin; PP1, protein phosphatase 1; Bdnf, brain-derived neurotrophin factor; CREB, cyclic-AMP response element binding protein; MeCP2, methyl-CpG binding protein 2; BAD, Bcl2 associated death protein; GSK3β, glycogen synthase kinase 3β; NMDAR, N-methyl-D-aspartate receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; VGCC, voltage-gated calcium channel; ApoER2, apolipoprotein E receptor 2; VLDLR, very low-density-lipoprotein receptor; DAB1, Disabled-1; SFK, Src family tyrosine kinase,. RELN, Reelin. Minus signs in red denote pathways that contribute to disease manifestation.

Table 1. Genes with altered DNA methylation and/or expression in AD

Process	Gene	Function	Significance	Modification	Effect	Reference
Amyloidogenic pathway	*App*	Amyloid precursor protein; Formation of Aβ plaque following cleavage in the amyloidogenic pathway	Spatial learning and memory deficits	Global DNA methylation	↓	31, 33
				Promoter methylation	--	29, 30
				Promoter methylation	↓	25, 27
				Exonic methylation	↑	28
				mRNA expression	↑	26
	Bace1	β-secretase 1; Cleavage of APP at the β site in the amyloidogenic pathway	Spatial learning and memory deficits; Its promoter methylation status is negatively correlated with the degree of cognitive decline	mRNA expression	↑	35
				Promoter methylation	↓	33
				Enhancer methylation	↓	36
	Psen1	Presenilin 1; Encode a core protein of γ-secretase	Spatial learning and memory deficits	Promoter methylation	↓	46
				mRNA expression	↑	47
	SORL1	Sortilin-related receptor 1; Direct APP holoprotein to recycling pathway; Endosomal Vesicle cycling	Degree of promoter methylation is associated with Aβ plaque and tau pathology	Promoter methylation	↑	55
				mRNA expression	↓	56
	Nep	Neprilysin; An Aβ-degrading enzyme facilitates Aβ clearance	Overexpression of NEP attenuates Aβ pathology	Promoter methylation	↑/--	31, 59
DNA methylation	*Dnmt1*	DNA methyltransferase 1	Maintenance of DNA methylation	mRNA expression	↓	11, 12
	Dnmt3a	DNA methyltransferase 3a	*De novo* DNA methylation			12
	Dnmt3b	DNA methyltransferase 3b				12
Neurochemical process	*Arc*	Activity-regulated cytoskeleton-associated protein;	Synaptic plasticity	mRNA expression	↓	74, 88, 97
					↑	89, 95, 96
	Egr1	Early growth response	Synaptic plasticity; Recruits TET1	mRNA	↓	74

	protein; transcriptional regulator	to facilitate demethylation	expression	↑	80
Bdnf	Brain-derived neurotrophic factor;	Synaptic plasticity; Neurogenesis	Promoter methylation	↑	116, 117
			mRNA expression	↓	98, 115
Npas4	Neuronal PAS domain protein 4; transcriptional regulator	Facilitates *Bdnf* and other activity-regulated gene transcriptions	mRNA expression	↓	122
Gsk3β	Glycogen synthase kinase 3β; phosphorylation of tau protein	Its association with NFT is more apparent in the initial disease stage	Promoter methylation	↓	125
			mRNA expression	↑	
Reln	Reelin; dendritic spine morphology; synapse development	Its transcription can be modulated by methionine supplement	Promoter methylation	--	132
			mRNA expression	↓	130, 131
Caln	Calcineurin; intracellular calcium homeostasis;	Memory-suppressing gene	Nuclear protein expression	↑	150
Pp1	Protein phosphatase 1;	Memory-suppressing gene; association with glutamatergic receptor attenuates synaptic transmission	Protein activity	↓	154
			mRNA expression in NFT	↓	88

↑, increase; ↓, decrease; --, unchanged



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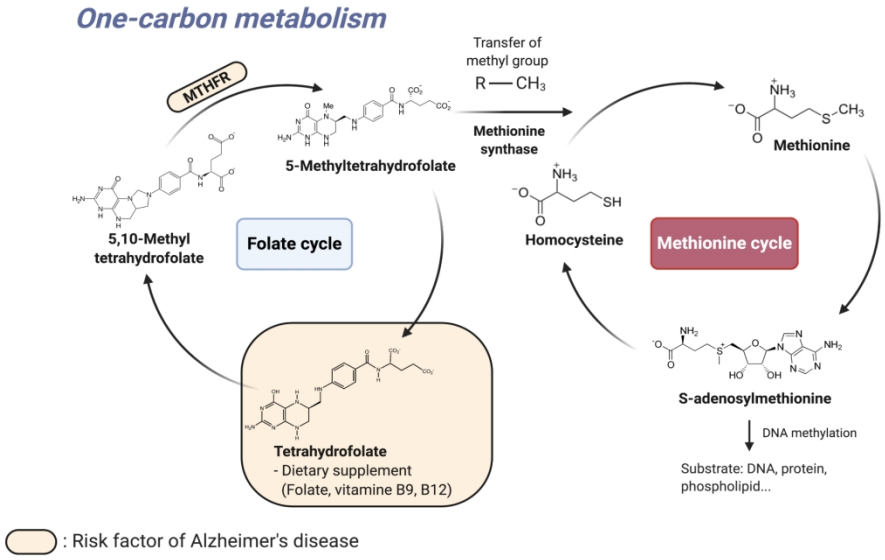
764x529mm (72 x 72 DPI)

Fig. 2



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MTHFR, methylenetetrahydrofolate reductase

764x529mm (72 x 72 DPI)

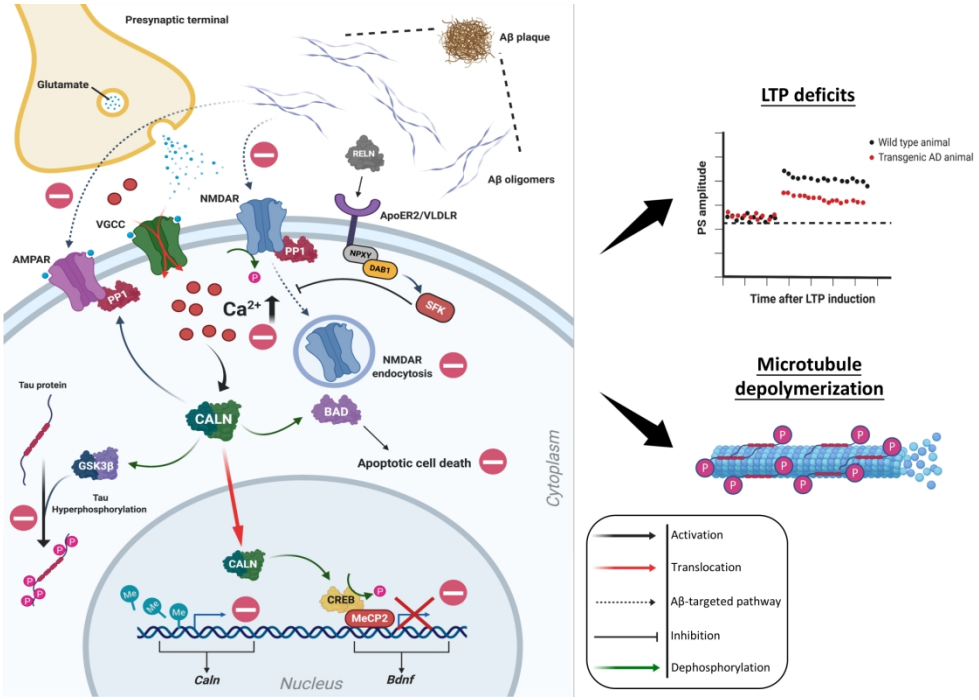

Figure 3. Proposed model of synaptic dysfunction in AD. Increased CALN expression and activity were observed in transgenic AD animal models. Impaired calcium homeostasis is considered to be one of the factors for elevated CALN activity in AD. Given the wide variety of its downstream substrates, CALN is thought to mediate tau protein hyperphosphorylation, PP1-mediated dephosphorylation of AMPAR and NMDAR, and BAD-dependent apoptosis and subsequent neuronal loss. PP1 associates with and dephosphorylates both AMPAR and NMDAR, decreasing synaptic transmission. Dephosphorylation of subunits in NMDAR is also associated with endocytosis of this receptor, thereby attenuating synaptic strength. The endocytosis of NMDAR may be rescued by reelin, which binds to ApoER2 to activate a downstream signaling cascade via SFK to enhance NMDAR subunit phosphorylation. Furthermore, both nuclear CALN and PP1 were shown to dephosphorylate, and hence inactivate CREB, which may result in transcriptional repression of Bdnf. This may further exacerbate the synaptic dysfunction in AD. GSK3β is activated upon dephosphorylation of CALN, which facilitates increased tau protein phosphorylation and contributes to microtubule depolymerization.

CALN, calcineurin; PP1, protein phosphatase 1; Bdnf, brain-derived neurotrophin factor; CREB, cyclic-AMP response element binding protein; MeCP2, methyl-CpG binding protein 2; BAD, Bcl2 associated death protein; GSK3β, glycogen synthase kinase 3β; NMDAR, N-methyl-D-aspartate receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; VGCC, voltage-gated calcium channel; ApoER2, apolipoprotein E receptor 2; VLDLR, very low-density-lipoprotein receptor; DAB1, Disabled-1; SFK, Src family tyrosine kinase, RELN, Reelin. Minus signs in red denote pathways that contribute to disease manifestation.

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