Review

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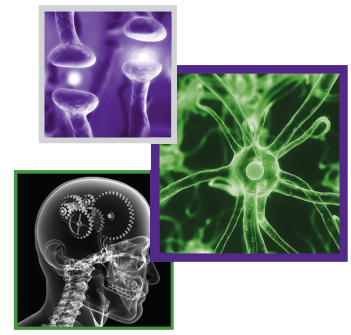
Neurodegenerative Disease Management



Role of epigenetics in Alzheimer's disease pathogenesis

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Practice points

- Alzheimer's disease (AD) is the most common type of dementia with several identified genetic and environmental risk factors.
- Epigenetic mechanisms, that mediate heritable changes in gene expression without changes in DNA sequence, are very sensitive to environmental stimuli.
- Several lines of evidence showed that the main epigenetic mechanisms, including DNA methylation, histone modifications and noncoding RNAs, could play an important role in AD pathogenesis.
- Pathological consequences of aberrant epigenetic modifications in the brain of AD patients are still little understood, and the main question in this field is if the observed modifications are cause or consequence of the neurodegenerative process.
- Epigenetic investigations performed in peripheral blood DNA of AD patients led to encouraging results in the search of peripheral biomarkers of the disease, but most robust studies are needed to confirm the preliminary findings.
- Studies performed in mitochondrial DNA from both brain and peripheral blood of AD and control subjects suggest a contribution of impaired mitochondrial DNA methylation in AD, a topic that deserves further investigation in AD and other neurodegenerative disorders.
- Studies performed in cell cultures and in animal models of AD provided evidence that targeting the epigenome with compounds exerting epigenetic properties could represent a promising preventative and therapeutic approach for neurodegeneration, but most should be done prior to translate those findings into the clinical settings.

Advances in molecular biology technologies have allowed uncovering the role of epigenetic regulation in several complex diseases, such as cancer and neurodegenerative disorders. Although the role of epigenetic mechanisms in Alzheimer's disease is still little understood, recent findings clearly show that such mechanisms are dysregulated during disease progression, already in its early stages. However, it is not clear if the observed epigenetic changes represent a cause or a consequence of the disease. Promising results are emerging from studies performed in peripheral blood DNA that could provide early biomarkers of the pathology. Moreover, given the dynamic nature of the epigenetic marks, intense research is carried out to investigate the therapeutic efficacy of compounds exerting epigenetic properties.

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Alzheimer's disease (AD) is the most common neurodegenerative disorder and the leading form of dementia worldwide [1]. The disease is characterized by memory loss and progressive cognitive decline resulting from neuronal death in several brain regions, including hippocampus, entorhinal areas, temporal and parietal lobes and restricted regions within the frontal cortex and the cingulate gyrus [1]. Amyloid plaques composed by extracellular aggregates of the amyloid β (Aβ) peptide and intracellular neurofibrillary tangles, which result from aggregates of hyperphosphorylated tau protein, are the main pathological hallmarks of the disease [2]. Mutations in three genes

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encoding for proteins involved in the production of the Aβ peptide, namely *APP*, *PSEN1* and *PSEN2* genes, lead to early-onset AD affecting individuals before the age of 65 years. However, *APP*, *PSEN1* and *PSEN2* mutations only explain a minority of early-onset AD, overall accounting for only about 1% of the total AD cases [3]. Indeed, more than 95% of AD is diagnosed in people over the age of 65 years, is sporadic in nature and defined as late onset AD (LOAD) [3].

To understand the etiology of LOAD, several researchers have focused on the identification of DNA sequence variants related to this condition. Until now, the *ϵ4* allele of the *APOE* gene represents the major genetic risk factor for LOAD, which however is neither necessary nor sufficient for the development of the disease [4]. Genome-wide association studies have revealed more than 20 susceptibility LOAD loci, mainly related to genes encoding for proteins involved in inflammation, cholesterol metabolism and endosomal vesicle recycling pathways, although each has a relatively low effect in the overall disease risk [1]. Furthermore, studies in monozygotic twins have revealed a variable concordance rate for AD, suggesting that risks factors other than genetic factors are involved in disease development [3]. Indeed, approximately a third of AD has been attributed to seven potentially modifiable risk factors, including diabetes, midlife hypertension and obesity, smoking, depression, cognitive inactivity and low educational attainment [5]. Moreover, several environmental factors including pesticides, metals, head injuries, lifestyles and dietary habits have been linked to LOAD risk [6]. Thus, LOAD is likely a complex disorder caused by complex interactions among genetic and nongenetic factors.

In this context epigenetic mechanisms, that are able to mediate the interaction between the genome and the environment, could provide a mechanistic explanation that might help our understanding of AD pathogenesis. This article provides a summary and a critical discussion of the main findings of studies addressing the role of epigenetics in AD.

Brief overview of major epigenetic mechanisms

The term epigenetics refers to heritable changes in gene expression that cannot be explained by changes in DNA sequence. Particularly, the epigenetic code is tissue and cell specific, and may change overtime as a result of aging, disease or environmental stimuli [7]. Indeed, a unique characteristic that differentiates epigenetic from genetic variation is that epigenetic processes are more responsive to the environment [8]. The main epigenetic mechanisms include DNA methylation, histone modifications and the regulation of gene expression mediated by noncoding RNA molecules (Figure 1).

DNA methylation is the most widely studied epigenetic mechanism and consists of the addition of a methyl group to the DNA, mediated by enzymes called DNA methyltransferases. The best-characterized DNA methylation process is the addition of a methyl group to cytosine in a CpG dinucleotide context, forming 5-methylcytosine (5-mC) [9]. Sites of CpG clusters are called CpG islands, and when a CpG island in the promoter region of a gene is methylated the expression of that gene is repressed. By contrast, cytosine methylation in gene bodies could be related to both active or repressed transcriptional state depending on the tissue in which it happens [10]. CpG dinucleotides are also located in repetitive or centromeric sequences, where their methylation is associated with the maintenance of chromosomal stability and with prevention of translocation events [11]. Hydroxymethylcytosine (5-hmC) is another modification of cytosine resulting from the oxidation of 5-mC mediated by members of the 10–11 translocation protein family. The CNS is peculiarly enriched in 5-hmC, and this epigenetic mark is likely to be involved in neurodevelopmental and neurodegenerative disorders [12].

Histones are the most abundant proteins associated with DNA, and aggregate each other forming the histone octamer around which DNA is wrapped creating the nucleosome. The N-terminal tails of histones may undergo several post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination and ADP ribosylation. These changes influence the chromatin structure, facilitating or inhibiting gene transcription [13]. For example, acetylation of lysine residues leads to a more relaxed chromatin structure, allowing greater access of transcriptional activators to the underlying genomic sequence [13].

In addition to histone modifications and DNA methylation, a further layer of epigenetic regulation of gene expression and chromatin state exists at the level of short (<200 nt) and long (>200 nt) nonprotein coding RNAs (ncRNAs) [14]. miRNAs (22–25 nt) are the most studied ncRNAs, and regulate gene expression in a sequence-specific manner. In fact they bind to the 3′ untranslated region of target mRNA molecules and mediate their post-translational regulation, leading to either degradation or translational inhibition, depending on the degree of sequence complementarity [15].

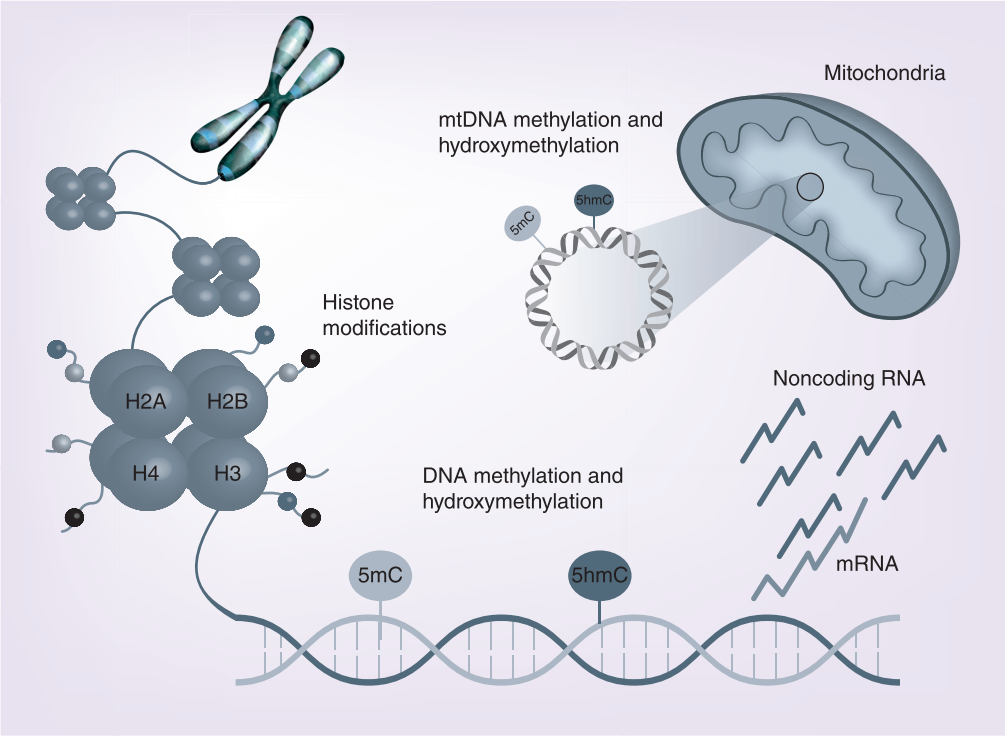

Figure 1. The main epigenetic mechanisms investigated in Alzheimer's disease pathology include DNA methylation, histone modifications and regulation of gene expression by noncoding RNAs. Recent findings indicate that also epigenetic changes of the mtDNA have a physiological role in cell function, and that dysregulation of mtDNA methylation could be involved in Alzheimer's disease pathogenesis.
mtDNA: Mitochondrial DNA.

DNA methylation in AD

The first evidence supporting a role for differential DNA methylation in AD pathogenesis was the observation of lower methylation levels of the *APP* promoter region in the temporal lobe of an AD patient respect to a nondemented subject and to a patient with Pick's disease [16]. Afterward, studies in neuronal cell cultures and animal models suggested that certain early-life environmental perturbations, including deficiency of B-group vitamins or lead exposure, induced methylation changes in genes required for Aβ peptide production [17–19]. Following those observations, several researchers have investigated DNA methylation in AD samples, at either global or gene specific level (Table 1).

Global DNA methylation has been evaluated as either 5-mC or 5-hmC content, or as the methylation level of repetitive elements, but studies in AD tissues have often produced conflicting results [20–30], so that it is difficult to understand the exact role of these epigenetic signatures in AD pathogenesis. More recently, significant alterations in both 5-mC and 5-hmC content were detected in early stages of AD across multiple brain regions, suggesting that global changes in DNA methylation and hydroxymethylation may play an early role in the progression of dementia [31].

The search for gene-specific methylation changes in postmortem AD brain samples was initially focused on dementia-related genes, including those required for the production of the Aβ peptide (*APP*, *PSEN1*, *PSEN2* and *BACE1*), involved in neurofibrillary tangle formation (*MAPT* and *GSK3B*), or associated to LOAD forms, such as *APOE* [16,32–34]. Collectively those studies showed no clear evidence of an altered epigenetic pattern of these genes in AD brain regions, and similar results were obtained from studies in blood DNA of living AD patients [35,36].

Several other genes involved in LOAD susceptibility, neuronal function, synaptic plasticity, inflammation or other AD-related pathways, have been investigated as potential epigenetic biomarkers of the disease in either blood or neuronal DNA samples [25,37–40]. Unfortunately, most of these studies are limited in sample-size, and results are often conflicting or lack replication, so that the clinical utility of those biomarkers is still uncertain [60,61]. However, among the several studied genes, *TREM2* and *BDNF* have been replicated in both brain and peripheral

Table 1. Overview of DNA methylation studies in Alzheimer's disease.

Experimental model	Method	End point investigated	Observation	Ref.
Neuronal cell cultures (SK-N-SH and SK-N-BE)	PCR-based methylation assay	*PSEN1* promoter methylation	B vitamin deprivation induced demethylation of *PSEN1* gene promoter, upregulation of PSEN1 and BACE1 proteins and increased Aβ production	[17]
Frontal cortical tissue of female monkeys (*Macaca fascicularis*)	DNA methyltransferase assay	DNMT activity	Early life exposure to lead (Pb) resulted in inhibition of DNMT activity and increased both *APP* and *BACE1* gene expression	[18]
Brain tissue of C57BL/6 mice	Western blot analysis and RT-PCR	Quantification of epigenetic enzymes	Cerebral levels of DNMT1 and MeCP2 were significantly reduced in mice exposed to lead (Pb) in early life	[19]
Temporal neocortex of monozygotic twins discordant for AD	Immunohistochemistry	5-mC levels	Reduction of 5-mC levels in brain regions of the AD twin	[20]
Entorhinal cortex of AD and control subjects	Immunohistochemistry	5-mC levels	Reduction of 5-mC levels in brain regions of AD patients	[21]
Hippocampus of AD and control subjects	Immunohistochemistry	5-mC and 5-hmC levels	Robust decrease in hippocampal levels of both 5-mC and 5-hmC in AD patients	[22]
Entorhinal cortex and cerebellum of AD and control subjects	Immunofluorescence	5-hmC levels	Decreased levels of 5-hmC in AD brain regions	[23]
Middle frontal gyrus and middle temporal gyrus of AD and control subjects	Immunohistochemistry	5-mC and 5-hmC levels	Both 5-mC and 5-hmC levels were significantly increased in AD brain regions	[24]
Frontal cortex of AD and control subjects	Immunohistochemistry and PCR-based methylation assay	5-mC levels and gene specific DNA methylation levels	Increased 5-mC levels in AD brain regions. *COX-2* and *NFK2B* hypomethylation, *BDNF* and *CREB* hypermethylation (AD vs control brains)	[25]
Hippocampus of AD, preclinical AD and control subjects	Immunohistochemistry	5-mC and 5-hmC levels	Increased levels of 5-mC and 5-hmC in hippocampal regions of both preclinical AD and AD patients	[26]
Entorinal cortex of AD and control subjects	Immunohistochemistry	5-mC and 5-hmC levels	No differences in 5-mC and 5-hmC levels between AD and control subjects	[27]
Peripheral blood of AD and control subjects	Pyrosequencing	Methylation levels of repetitive elements (Alu, LINE-1 and SAT-a sequences)	Increased LINE-1 methylation levels in AD subjects with respect to controls	[28]
Peripheral blood of AD and control subjects	Luminometric methylation assay	Methylation of CCGG sites	Increased global methylation of CCGG sites in AD samples	[29]
Peripheral blood of AD and control subjects	MS–HRM	Methylation levels of repetitive elements (LINE-1)	No differences in LINE-1 methylation levels between AD and control subjects	[30]
Several brain regions of AD, preclinical AD and control subjects	Gas chromatography/mass spectrometry (GC/MS)	5-mC and 5-hmC levels	Significant alterations in both 5-mC and 5-hmC levels in early stages of AD	[31]
Temporal lobe of one AD and one control subjects	Enzyme digestion (HpaII) and southern blot	*APP* gene methylation	Hypomethylation of *APP* in the AD subject	[16]
Frontal cortex and hippocampus of AD and control subjects	MALDI-ToF	DNA methylation of several genes including *MAPT*, *APP* and *PSEN1*	No differences in the methylation levels of the analyzed genes between AD and controls	[32]
Brain tissue (cerebellum, anterior parietal lobe and inferior temporal lobe) of AD and control subjects	Pyrosequencing	DNA methylation of several genes, including *ACE, APOE, APP, BACE1, GSK3B, MAPT* and *PSEN1*	Hypermethylation of *APP*, *MAPT* (in both neuronal and non-neuronal cells), and of *GSK3B* (in non-neuronal cells) in AD subjects	[33]
Brain tissue and blood lymphocytes of AD and control subjects	MALDI-ToF	DNA methylation of several genes, including *MTHRF, DNMT1, BACE1, APP, PSEN1* and *APOE*	AD brains showed an increased epigenetic drift with unusual methylation patterns, particularly concerning *PSEN1*, *APOE*, *MTHFR* and *DNMT1* genes	[34]
Peripheral blood of AD and control subjects	Pyrosequencing	*APP*, *PSEN1* and *PSEN2* methylation	Increased methylation of *APP* and *PSEN1* in AD samples	[35]
Peripheral blood of AD and control subjects	MS–HRM	DNA methylation analysis of several genes, including *PSEN1*, *BACE1*, *DNMT1*, *DNMT3A*, *DNMT3B* and *MTHFR*	No differences were observed in the methylation levels of the analyzed genes between AD and controls	[36]
Temporal lobe of AD and control subjects	Illumina infinium 450 K methylation array	The array covered several selected AD-related loci	*ABCA7, CASS4, CELF1, CD33, EPHA1, FERMT2, MEF2C, PTK2B, SORL1* and *ZCWPW1* genes differentially methylated between AD and control tissues	[37]

5-hmC: Hydroxymethylcytosine; 5-mC: 5-methylcytosine; AD: Alzheimer's disease; aMCI: Amnestic mild cognitive impairment; EWAS: Epigenome-wide methylation and hydroxymethylation study; MALDI-ToF: Matrix-assisted laser desorption ionization time-of-flight; MS–HRM: Methylation sensitive-high resolution melting.

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Table 1. Overview of DNA methylation studies in Alzheimer's disease (cont.).

Experimental model	Method	End point investigated	Observation	Ref.
Brain tissue from AD and control subjects	Illumina infinium 450 K methylation array	The array covered several selected AD-related loci	Increased DNA methylation of *SORL1, ABCA7, HLA-DRB5, SLC24A4* and *BIN1* associated with AD.	[38]
Frontal cortex of AD and control subjects	Illumina VeraCode GoldenGate DNA methylation array	The array covered several selected AD-related loci	Hypermethylation of *TBXA2R, SORBS3* and *SPTBN4* genes in AD samples	[39]
Hippocampus of AD and control subjects	Bisulfite sequencing	DNA methylation analysis of *CRTC1*	Methylation levels of *CRTC1* were lower in AD than in controls	[40]
Hippocampus of AD and control subjects	Bisulfite sequencing and hMeDIP	DNA methylation and hydroxymethylation analysis of *TREM2*	Increased *TREM2* methylation in AD samples compared with controls	[41]
Superior temporal gyrus of AD and control subjects	Pyrosequencing	*TREM2* methylation	Hypermethylation of *TREM2* in AD subjects	[42]
Peripheral blood of AD and control subjects	Pyrosequencing	DNA methylation analysis at four CpG sites in intron 1 of *TREM2*	Reduced methylation of *TREM2* intron 1 in AD samples.	[43]
Peripheral blood of AD and control subjects	Pyrosequencing	DNA methylation of four CpG sites in the promoter of the *BDNF* gene	*BDNF* methylation was significantly higher in AD samples than in controls	[44]
Peripheral blood of AD and control subjects	Bisulfite sequencing	DNA methylation analysis of the *BDNF* gene promoter	*BDNF* methylation was significantly higher in AD samples than in controls	[45]
Peripheral blood of AD, aMCI and control subjects	Pyrosequencing	DNA methylation analysis of the *BDNF* gene promoter	Increased *BDNF* methylation levels in aMCI subjects	[46,47]
Frontal cortex of AD and control subjects	Illumina infinium bead array	EWAS	Identified 948 differentially methylated CpG sites between AD and control subjects	[48]
Superior temporal gyrus of AD and control subjects	Illumina infinium 450 K methylation array	EWAS	Identified 479 differentially methylated CpG sites between AD and control subjects	[49]
Cortical brain human tissue	Illumina infinium 450 K methylation array	EWAS	Identified 71 differentially methylated CpG sites potentially associated with the burden of AD pathology	[50]
Human brain and peripheral blood samples	Illumina infinium 450 K methylation array	EWAS	Identified a differentially methylated region in the *ANK1* gene that was associated with neuropathology in the entorhinal cortex	[51]
Human AD brains	High-throughput sequencing	Genome-wide analysis of 5-hmC	Identified more than 500 differentially hydroxymethylated regions associated with AD neuropathology	[52]
Prefrontal cortex of AD and control subjects	High-throughput sequencing	Genome-wide analysis of 5-hmC	Identified 325 hydroxymethylated regions between AD and control brains	[53]
Peripheral blood of AD, aMCI and control subjects	Illumina infinium 450 K methylation array and pyrosequencing	EWAS	*NCAPH2/LMF2* promoter methylation was significantly decreased in both AD and aMCI samples	[54]
Peripheral blood of AD and aMCI	Pyrosequencing	Methylation analysis of *NCAPH2/LMF2* promoter	*NCAPH2/LMF2* methylation levels correlated with hippocampal atrophy	[55]
Peripheral blood of AD and control subjects	Immunohistochemistry	5-mC levels	No difference in 5-mC levels between patients and controls. However, 5-mC levels were associated with markers of folate status	[56]
Peripheral blood of AD and control subjects	MS–HRM	Methylation levels of *PSEN1, BACE1, DNMT1, DNMT3A, DNMT3B* and *MTHFR*	Methylation levels of *PSEN1, BACE1, DNMT1, DNMT3A, DNMT3B* and *MTHFR* were linked to circulating levels of folate, homocysteine and vitamin B12	[57]
Entorhinal cortex of individuals with AD-related pathology and control subjects	Pyrosequencing	Methylation analysis of the mitochondrial D-loop region and of *MTND1* and *MTND6* mitochondrial genes	Increased D-loop methylation in individuals with AD-related pathology	[58]
Peripheral blood of AD and control subjects	MS–HRM	Methylation analysis of the mitochondrial D-loop region	Decreased methylation of the D-Loop region in AD patients	[59]

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blood samples of AD, likely representing potential methylation biomarkers of the disease [25,41–47]. Furthermore, *BDNF* promoter methylation was able to predict the conversion from amnestic mild cognitive impairment (aMCI, a condition frequently seen as a prodromal stage of AD) to AD [46,47].

Candidate-gene approaches have been paralleled in recent years by epigenome-wide methylation and hydroxymethylation studies (EWAS). Those studies have revealed hundreds of differentially methylated (DMRs) or hydroxymethylated (DhMRs) regions between postmortem AD and control brains [48,51]. The most replicated DMRs in EWAS studies are associated with eight genes, including *LOC100507547*, *PRDM16*, *PPT2*, *PPT2-EGFL8*, *PRRT1*, *C10orf105*, *CDH23* and *RNF39*, which encode for proteins involved in several cellular pathways such as regulation of gene expression, synaptic plasticity and intercellular communication [49–51,62]. For what is concerning DNA hydroxymethylation, a recent genome-wide investigation of postmortem AD samples (dorsolateral prefrontal cortex), revealed hundreds of DhMRs associated with disease neuropathology, including 517 DhMRs significantly associated with neuritic plaques and 60 with neurofibrillary tangles [52]. A similar study in DNA extracted from the prefrontal cortex of postmortem AD patients identified 325 genes containing DhMRs in both discovery and replication datasets [53].

Collectively, despite that the results are sometimes conflicting, most of the current literature suggests several methylation and hydroxymethylation differences between AD and control samples. Some correlations between AD pathological hallmarks and gene specific methylation changes were also observed. For example, promoter methylation levels of the *NCAPH2/LMF2* gene correlated with hippocampal atrophy in both AD and aMCI individuals [54,55], and there are some reports linking circulating folate levels in AD patients with either global or gene specific methylation changes in blood DNA [56,57].

Mitochondrial epigenetics & AD

In addition to the nuclear DNA, increasing evidence is showing that also the mitochondrial DNA (mtDNA) could be regulated by epigenetic mechanisms [63]. Mitochondrial impairment is a recurrent feature of AD and could be partially explained by a dysregulation of mtDNA epigenetic mechanisms [64]. An initial study in this field showed a nonsignificant increase of global 5-hmC levels in the mtDNA extracted from the temporal gyrus of AD patients respect to healthy controls [26]. More recently, an increased methylation of the mitochondrial displacement loop (D-loop) region, a region critical for mtDNA replication and transcription, was observed in postmortem DNA from the entorhinal cortex of eight patients with AD-related pathology. The degree of D-loop methylation was higher in early disease stages, and a dynamic pattern of methylation was observed in brain regions of transgenic AD mice [58]. A subsequent analysis of blood DNA samples from 133 living LOAD patients and 130 matched controls, revealed a significant 25% reduction of D-loop methylation levels in the first group, thus strengthening previous evidence that epigenetic modifications of the mtDNA might occur in neurodegenerative diseases [59].

Histone tail modifications in AD

Data on histone tail modifications in human tissues of AD patients (Table 2) suggest that several of them could occur in AD brain and peripheral blood, including phosphorylation [25], acetylation [65–67] and methylation [68]. Interestingly, the investigation of brain regions of two transgenic mouse models of AD revealed an early increase in the acetylation of histone H4 at lysine 12 (H4K12ac), that occurred during the development of the amyloid aggregates in the brain [69]. H4K12ac was also increased in monocytes of aMCI patients, but not in patients with AD, suggesting that it could represent an early event in AD development [69].

A recent genome-wide methylation study in AD brains revealed that the identified DMRs overlapped promoters marked by two histone modifications, namely H3K27me3 and H3K4me3, suggesting that these epigenetic mechanisms work in concert to regulate gene expression levels [49].

In this regard, several studies have been performed in neuronal cell cultures and animal models of AD in order to evaluate the potential role of epigenetic drugs, including inhibitors of HDACs (HDACi) and methyl donor compounds, to counteract neuropathology and cognitive decline (reviewed in [70]). Indeed, many investigators reported that transgenic AD mice treated with HDACi, such as sodium butyrate, trichostatin A or valproic acid, showed an improvement of learning and memory [70]. Furthermore, it was demonstrated that certain HDACi were able to decrease Aβ levels in transgenic mice by targeting genes required for Aβ formation [71], and that the selective inhibition of HDAC2 unlocked the repression of genes related to learning and memory [66]. Several of these compounds are however toxic and nonspecific, and there is increasing interest in natural molecules exerting

Table 2. Main findings on histone tail modifications in Alzheimer's disease.

Experimental model	Method of measurement	End point investigated	Observation	Ref.
Frontal cortex of AD and control subjects	Immunohistochemistry	Global histone tail modifications	Increased histone 3 (H3) phosphorylation in AD samples	[25]
Temporal lobe of AD and control subjects	Mass spectrometry	Histone tail acetylation	Histone acetylation (H3 K18/K23) was significantly lower in AD samples	[65]
Brain tissue of AD and control subjects; AD animal models	Western blot, chromatin immunoprecipitation	HDAC2 levels	Increased HDAC2 levels in both AD samples and animal brains. The animal models revealed that HDAC2 regulates the expression of memory-related genes	[66]
Middle temporal gyrus of AD and control subjects	Immunohistochemistry	Histone tail acetylation	Significant increase in histone tail acetylation (H3ac and H4ac) in AD samples	[67]
Frontal cortex of AD and control subjects	Mass spectrometry	Several histone tail modifications	Decreased levels of H2BK108 and H4R55 methylation, increased levels of H2BK120 ubiquitination and loss of H4 N-terminus acetylation were observed in AD samples	[68]
Monocytes of two transgenic AD mouse models and monocytes of both MCI and AD individuals	Immunohistochemistry	Acetylation of H4K12	Increased H4K12 acetylation was observed in both transgenic mouse models during plaque deposition in the brain. Increased H4K12 acetylation was observed in MCI but not in AD patients	[69]

AD: Alzheimer's disease; MCI: Mild cognitive impairment.

epigenetic properties, including dietary B-vitamins, resveratrol, curcumin, epigallocatechin-3-gallate and many more, that could represent useful and safer compounds in the treatment of AD-related cognitive decline [70].

Noncoding RNAs & AD

Accumulating evidence suggests that alterations in the ncRNA network contribute to AD pathogenesis (Table 3). Most of these studies investigated the contribution of miRNA dysregulation to AD pathogenesis, but also long noncoding RNAs (lncRNAs) are emerging as possible epigenetic players in AD development [72].

Regarding miRNAs, several authors evaluated the expression profiles of those regulating genes required for the production of the Aβ peptide or involved in the formation of protein tau aggregates [73–80]. MiRNAs belonging to the miR-29 family are the best characterized in the regulation of *BACE1,* a gene encoding the rate-limiting enzyme in Aβ peptide generation, and have been found to be dysregulated in brain and peripheral blood tissues of AD patients [73,79,80]. It was also observed that miR-29c promotes learning and memory processes in hippocampal neurons, and could represent a potential therapeutic target in AD [80]. Several other miRNAs involved in lipid metabolism, such as miR-33, and in neuroinflammation, such as miR-34a and miR-155, have been proposed as important regulators in AD pathogenesis [81–83].

In addition to miRNAs, other ncRNAs have been related to AD pathogenesis. For example, lncRNAs involved in synaptic plasticity or apoptosis [84,85] and in the production of the Aβ peptide [86–89] have been linked to AD. A recent analysis of published microarray data from postmortem brains revealed 24 upregulated and 84 downregulated lncRNAs in AD brain regions [90]. Additional lncRNAs were found to be dysregulated in AD brains by RNA sequencing analysis [91]. Also circular RNAs and Y RNAs, a family ncRNAs of about 100 nucleotides in length, are emerging as molecules with potential roles in AD [92,93].

Taken together these observations suggest that ncRNAs could contribute to the regulation of AD-related genes. Indeed, several researchers are trying to develop ncRNA-based methods to threat AD, and to identify peripheral ncRNAs able to detect the disease in early stages [72]. Although results obtained so far are promising, additional research is needed [72].

Conclusion

Epigenetic modifications have been largely documented in affected brain regions and in peripheral lymphocytes of individuals affected by AD, suggesting their contribution to disease development and progression. However, the biological significance of the epigenetic changes observed so far in affected brain areas is still largely debated, mainly due to the conflicting nature of the findings. Several factors may have contributed to discrepancies in the results obtained by different investigators, such as the use of different methods to assess methylation levels, the often limited sample size of studied populations and the different areas of the brain, as well as the cellular types investigated [60].

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Table 3. Main findings on noncoding RNA expression in Alzheimer's disease.

Experimental model	Method of measurement	End point investigated	Observation	Ref.
Brain tissue of AD and control subjects and cell cultures	MiRNA microarray	Expression profiles of several miRNAs	The miR-29a/b-1 cluster was significantly decreased in AD patients displaying high BACE1 protein levels. MiR-29a/b-1 regulate BACE1 expression *in vitro*	[73]
Brain tissue of AD and control subjects and cell cultures	Real-time quantitative PCR	Expression profiles of several miRNAs	miRNAs belonging to the miR-20a family, including miR-20a, miR-17–5p and miR-106b regulate *APP* expression *in vitro*. A significant reduction in miR-106b expression was observed in AD tissues	[74]
Animal models and cell cultures	Real-time quantitative PCR	Expression levels of miR-195	MiR-195 inhibits the translation of BACE1	[75]
Brain tissue of AD and control subjects and cell cultures	Real-time quantitative PCR	Expression levels of miR-26b	Elevated levels of miR-26b were observed in AD samples. Overexpression of miR-26b in rat primary neurons led to increased tau-phosphorylation	[76]
Human cell cultures	Real-time quantitative PCR	Expression levels of miR-144	MiR-144 suppresses α-secretase (ADAM10) expression	[77]
Mouse cell cultures	Luciferase activity assay and western blot analysis	Effect levels of miR-9 on Aβ42 formation	MiR-9 attenuated Aβ-induced synaptotoxicity by targeting the calcium/calmodulin-dependent protein kinase kinase 2 (CAMKK2)	[78]
Brain tissue of AD and control subjects and cell cultures	Real-time quantitative PCR	Expression levels of miR-29c	MiR-29c was downregulated in AD samples in association with BACE1 upregulation	[79]
Peripheral blood of AD and control subjects	Real-time quantitative PCR	Expression levels of miR-29c	Marked reduction of miR-29c in AD samples	[80]
Mouse models	Real-time quantitative PCR and luciferase activity assay	Identification of brain lipid metabolism regulatory mechanisms	Inhibition of miR-33 increased lipidation of brain ApoE and reduced Aβ levels	[81]
Peripheral blood of AD and control subjects	MiRNA microarray and real-time quantitative PCR	Expression levels of several miRNAs	Several miRNAs resulted upregulated in AD samples, particularly miR-34a and miR-181b	[82]
Mouse models	Real-time quantitative PCR	Functional role of miR-155	Upregulation of miR-155 levels was observed in brain regions of AD mice before the appearance of extracellular Aβ aggregates	[83]
Various brain areas of AD and control subjects	Northern hybridization	Levels of lncRNA BC200	BC200 levels resulted significantly upregulated in AD samples	[84]
Primary cultures of rat cortical neurons	Real-time quantitative PCR	Expression of NAT-Rad18	NAT-Rad18 was upregulated during Aβ-induced apoptosis	[85]
Brain tissue of AD and control subjects, animal models and cell cultures	Sequencing	Analysis of the BACE1-AS	BACE1-AS regulates BACE1 mRNA and protein expression *in vitro* and *in vivo*. BACE1-AS levels resulted elevated in AD samples	[86]
Cell cultures and brain tissue of AD and control subjects	Real-time quantitative PCR	Functional role of ncRNAs in *SORL1* regulation	The ncRNA called 51A drives a splicing shift of the *SORL1* gene, a process associated to increased Aβ formation. 51A resulted overexpressed in AD samples	[87]
Cell cultures and brain tissue of AD and control subjects	Real-time quantitative PCR	Analysis of ncRNA 17A	The ncRNA 17A was overexpressed in AD samples. 17A expression in neuroblastoma cells enhanced Aβ peptide secretion	[88]
Cell cultures and frontal and temporal cortices from AD and control subjects	Real-time quantitative PCR	Expression of ncRNA NDM29	The ncRNA NDM29 induced APP synthesis with increased accumulation of Aβ peptide in cell cultures. An increased synthesis of NDM29 was observed in AD tissues	[89]
Brain tissue of AD and control subjects	Analysis of published datasets	Reannotation of microarray data	The study identified 24 upregulated and 84 downregulated lncRNAs in AD brain regions	[90]
Hippocampus of AD and control subjects	RNA sequencing and Real-time quantitative PCR	Expression profiles of several ncRNAs	Several lncRNAs were differentially expressed in AD brains (21 upregulated and ten downregulated)	[91]
Brain samples of AD and control subjects	Northern hybridization	Levels of miRNA-7-circRNA	Altered regulation of miRNA-7-circRNA system in AD brains	[92]
Brain samples of AD and control subjects	RNAseq analysis	Genome-wide RNA binding map of nELAVL RNA binding proteins	Significant changes of nELAVL RNA binding proteins on noncoding Y RNAs in AD samples	[93]

AD: Alzheimer's disease; BACE1-AS: BACE1-antisense transcript; lncRNA: Long noncoding RNA; nELAVL: ELAV-like.

In this regard, it is has recently been reported that different types of brain cells show different levels of methylation, even in the same brain area [94]. Given these drawbacks in the study of epigenetic modifications in postmortem brain samples, epigenetic changes have been extensively studied in easily accessible peripheral blood samples of subjects with AD, leading to interesting results with the potential to provide peripheral epigenetic biomarkers of disease progression [61]. Also in this case, however, results are still in their infancy. The data produced so far are interesting and encouraging, but differences in the panels of studied genes, the different methodological approaches, and the relatively low sample-size, have not yet allowed to produce robust peripheral epigenetic biomarkers of the disease [61]. Hopefully, the increasing number of well-designed and powered epigenome-wide investigations could have the potential to lead to more robust and replicated markers in the near future. In this regard, the use of peripheral tissues provides the opportunity to investigate epigenetic events associated with different disease stages and to follow the patients over time. However, it is still unclear to which extent DNA methylation alterations in peripheral blood actually reflect those occurring in the brain in that moment. A recent study performed in living patients with epilepsy that underwent neurosurgical treatment has revealed that only about 8% of the epigenetic changes observed in peripheral blood DNA reflect those observed in brain regions [95], suggesting that only a subset of the peripheral markers may reflect the methylation status of brain tissues, and something similar is likely to occur in other diseases.

Another issue in AD epigenetics is if the observed changes in postmortem tissues are cause or consequence of the disease. Some authors believe that the epigenetic insult has occurred in early-life, during neurogenesis and synaptic formation [18,19]. Other possibilities include life-long induced epigenetic modifications by dietary habits, lifestyles, as well as occupational and environmental exposures leading to age-related epigenetic drifts linked to dementia [34]. However, some or most of the observed epigenetic changes could be the result of the neurodegenerative process itself, and arise as a consequence of increased production of neurotoxic compounds, increased oxidative stress, hyperhomocysteinemia, reduced B-vitamins, inflammation and so on. One of main goals in future research will be our ability to discriminate the early epigenetic events leading to dementia from those contributing to its progression, with the aim to detect early disease biomarkers and preventive or therapeutic strategies [96]. Indeed, given that epigenetic modifications are reversible, the identification of either natural or synthetic compounds able to target the epigenome is one of the most promising strategies to counteract cognitive decline and neurodegeneration later in life [70,97].

Future perspective

Overall, it is clear that impaired epigenetic pathways play a critical role in AD pathogenesis, and the conflicting results reported in the literature highlight the need to perform better and more in-depth studies, including the analysis of larger cohorts of individuals as well as longitudinal studies. Technological improvements will allow to better investigating the epigenetic landscape in AD, by increasing the proportion of the genome that can be analyzed in a deeper manner. Furthermore, it will be possible to clarify the role of newly emerging epigenetic marks, such as 5-hmC, methylation in a non-CpG context (termed 5mCH, where H is an A, T, or C), as well as RNA methylation, that are appearing to have an important role in brain cell metabolism [10,98]. Moreover, recent evidence points to a potential contribution of impaired mtDNA methylation in AD and in other neurodegenerative diseases [58,59], a topic that needs further investigation given the pivotal role of mitochondria in neurodegeneration.

A key challenge in the study of epigenetic regulation in AD is to understand if epigenetic changes are a cause or an effect of the pathological process, or if both are true, in order to discriminate early events from later ones. In this regard, recent advancing in genome editing, with the use of the CRISPR/Cas9 technology, will permit to induce epigenetic changes in both *in vitro* and *in vivo* models of AD, thus allowing determining the functional consequences of such modifications [62,99]. Moreover, understanding the temporal modifications that underlie AD pathogenesis may provide new molecular targets for therapeutic interventions.

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