

# Epigenetic mechanisms in Alzheimer's disease

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**Abstract:** The worldwide increase in life expectancy is leading to an increase in age-dependent diseases, including nonfamilial, sporadic Alzheimer's disease (AD), which is the subject of this review. The etiology and pathophysiology of the disease is not fully understood, but present observations suggest that, in addition to genetic risk factors, environmental influences may be involved via epigenetic mechanisms. Currently, there is no effective treatment, but there are indications that lifestyle has an impact on the development of the disease. This view is supported by preclinical studies not only showing that human lifestyle-equivalent interventions have a positive effect on cognitive function in animal models of AD, but also indicating the involvement of underlying epigenetic mechanisms. After a brief overview of the most characteristic chromatin modifications, ie, DNA methylation and histone modifications, epigenetic changes associated with aging are considered, given that aging is the most important risk factor for AD. This is followed by a description of some epigenetic alterations recognized in AD. The impact of environmental factors and lifestyle on the epigenome is then considered. Epigenetic treatments with HDAC inhibitors and RNA-based drugs are considered, which – while still in preclinical stages – are promising for potential benefit. It is concluded that while awaiting results from clinical trials in progress, focusing on lifestyle adjustments with an epigenetic background are the best way to prevent/delay the onset of this devastating disease.

**Keywords:** Alzheimer's disease, epigenetic mechanisms, environmental causes

## Introduction

The definition of epigenetics has gone through some changes since its inception by Waddington, who described it as “the interaction of genes with the environment, which brings the phenotype into being”.<sup>1</sup> For Arthur Riggs, it meant “heritable changes in gene function that cannot be explained by changes in DNA sequence”. The heritable requirement would not apply to mature neurons, which do not divide, but epigenetic changes involving modification of chromatin do take place, causing changes in gene expression in response to environmental influences, for example, in underlying learning and memory processes.<sup>2</sup> Bird put forward a unifying definition of epigenetic events: “the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states.”<sup>3</sup> It is noteworthy that experimental studies have indicated that behavioral interventions or mimicking of human disease, including neurodegenerative disorders, induce changes in chromatin that are lasting but potentially reversible. It is understandable therefore that there is much interest in exploring epigenetic mechanisms involved in such diseases, because they raise hope for successful therapeutic

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interventions. One of these diseases is nonfamilial, late-onset Alzheimer's disease (AD), which is the subject of the present review.

## Alzheimer's disease

The most characteristic feature of AD is cognitive impairment that, together with neuropathological changes, extracellular amyloid plaques, intracellular neurofibrillary tangles, and loss of synapses and neurons, are similar in the early-onset and late-onset forms of the disease.<sup>4</sup> Both forms of AD have genetic components. Mutations in three genes identified in the early-onset familial form, ie, amyloid precursor protein (APP), presenilin-1, and presenilin-2, established the central role of amyloid in the disease.<sup>5</sup> Nevertheless, mutations in these genes are present in only 13% of patients with the early-onset form of the disease. Technological advances, such as large-scale genome-wide association studies, have led to identification of more than ten risk genes for the late-onset form of AD.<sup>4</sup> *Apolipoprotein E* is the most important risk gene, with one apolipoprotein E4 allele increasing the risk three-fold and two alleles increasing it 15-fold. The contribution of other susceptibility genes to the increased risk is much smaller, although rare variants with large effects have been identified.<sup>6,7</sup> While new technologies have changed the genetic landscape of AD, it is evident that environmental factors, the effects of which are mediated by epigenetic mechanisms, also play an important role in the pathogenesis.

## Epigenetic mechanisms

### DNA methylation

One of the best characterized chromatin modifications is DNA methylation. The human genome is predominantly methylated on CpG motifs. There are more than 50,000 CpG islands in the human genome. They are enriched in promoters, where their methylation results in gene silencing. However, the promoter methylation level is usually low and tissue-specific methylation is primarily found at the shores of CpG islands, within approximately 2 kilobases of the CpG-rich sequences.<sup>8</sup> Interestingly, methylation within the body of genes usually marks active transcription.<sup>9</sup>

DNA methylation is affected by DNA methyltransferases (DNMT). DNMT1 provides for the maintenance of existing marks, whereas DNMT3a and DNMT3b are de novo methylases. However, these distinctions are not stringent and DNMT1 can be involved in de novo methylation and the DNMT3s in maintenance. The level of DNMT1 and DNMT3a proteins decreases in the aged primate brain.<sup>10</sup>

DNA methylation changes in the brain are dynamic. This was indicated by the finding that changes in DNA methylation contribute to neuronal plasticity (for reviews see Zovkic et al<sup>2</sup> and Day and Sweatt<sup>11</sup>). Thus, although methylated cytosine is a very stable mark, there must also be a demethylating function that has escaped identification for a long time. The recent discovery of 5-hydroxymethyl cytosine (5-hmC) in the mammalian genome paved the way for understanding the mechanism of DNA demethylation.<sup>12,13</sup> 5-hmC is present in the brain at about ten-fold higher levels than in most peripheral tissues and accounts for about 40% of modified cytosine in the genome.<sup>13</sup> There is now evidence that 5-hmC is an intermediate in the DNA demethylation pathway.<sup>14-16</sup> 5-methylcytosine (5-mC) is oxidized with the help of the ten-eleven translocation (TET) enzymes, which are Fe<sup>2+</sup>-dependent and 2-ketoglutarate-dependent oxidases. Currently, three TET enzymes (TET1-3) are known. In addition to having a catalytic subunit with an iron-binding site, they contain a binuclear zinc-chelating CXXC domain that is found in many chromatin-interacting proteins. There is evidence that 5-hmC is involved in cellular development processes and that it is associated with pluripotency. TET1 and TET2 are important to maintain pluripotency, while TET3 is associated with differentiation processes. 5-hmC levels increase with age until they ultimately reach a stable plateau.<sup>13</sup> The current model of DNA demethylation involves TET-mediated oxidation of 5-mC, which may be followed by further oxidation to 5-formylcytosine and 5-carboxylcytosine (5-fC and 5-caC, respectively). 5-fC and 5-caC are then excised by a glycosidase and base-excision repair introduces a cytosine to fill the gap.

DNA methylation has been reviewed by Song and He,<sup>17</sup> who concluded that there is strong evidence for 5-hmC serving as a stable epigenetic mark, in addition to being an intermediate in demethylation. Further, although 5-fC and 5-caC levels are much lower compared with those of 5-hmC, they might also have some regulatory role. Mellen et al<sup>18</sup> studied the distribution and role of 5-hmC in three types of cells in the cerebellum, ie, Purkinje cells, granule cells, and Bergmann glia. These elegant studies used animals carrying an artificial bacterial vector driven by cell type-specific gene regulatory regions coupled to enhanced green fluorescent protein L1 that is concentrated in the nucleoli of the specific cell type, permitting isolation of cell type-specific nuclei by fluorescence sorting. In addition, the labeled polysomes permit isolation of messenger RNA (mRNA) and analysis of a cell-specific repertoire of transcripts using the translating ribosome affinity purification method<sup>19,20</sup> coupled with quantitative high-throughput sequencing of the isolated mRNA. 5-hmC was found to be enriched in active genes, where 5-mC was depleted and its

contribution to gene expression was cell type-specific. Of the various methyl-binding proteins, MeCP2 is selectively bound to 5-hmC with an affinity similar to that of MeCP2 binding to 5-mC. The association of MeCP2 with 5-hmC at expressed genes is interesting because MeCP2 has been considered to mediate a repressive influence. Nevertheless, it was observed that a deficiency of MeCP2 (causing the developmental disorder, Rett syndrome) leads to downregulation of gene expression, whereas high MeCP2 expression that also causes neuropsychiatric disorders, results in upregulation,<sup>21–23</sup> and the findings of Mellen et al<sup>18</sup> clarify this issue. This dual function of MeCP2 seems to depend on binding to 5-hmC, which is associated with gene activation, whereas binding to 5-mC leads to repression, and the role of MeCP2 appears to involve modulation of the organization of chromatin.

Methylated CpGs are targets for binding proteins. These include MeCP1 and MBD2, in addition to MeCP2. MeCP1 comprises ten separate peptides and acts as a mediator between DNA methylation and histone acetylation. MeCP1 is not bound directly to methylated DNA, but to MBD2 protein, that binds the complex to 5-mC, thereby inducing transcriptional repression.<sup>24</sup>

## Histone modifications

The basic structural elements of chromatin are nucleosomes, which comprise a histone core around which DNA is wrapped. The histone core is an octamer containing two units from H2A, H2B, H3, and H4, while the H1 linker is involved with packing of the bead-like nucleosomes into a higher order structure. There are many post-translational modifications on the histone tails, of which acetylation and methylation have been the most extensively studied. The acetylation mark indicates access of the transcription machinery to the genes and thus active transcription. The functions of histone methylation are more complex (for review, see Black and Whetstone<sup>25</sup>). Methylation marks many different states. For example, H3K4me3 is found at transcription start sites and correlates strongly with active gene expression, while H3K36me3 is found in the body of actively transcribed genes. In contrast, H3K9me3 correlates with repressed genes when found at the promoter. However, H3K9me3 within the body of a gene correlates with gene expression.

Different regions of chromatin are also characterized by different histone marks. H3K79me is enriched in euchromatin, whereas H3K9me3 and H4K20me3 largely occur in heterochromatin. In addition, other marks are associated with different events, such as phosphorylation of H2AX at

sites of DNA damage. Histone modifications function by providing docking sites for other proteins. For example, bromodomain-containing proteins, such as the SWI/SNF chromatin remodelers, bind acetylated lysines and heterochromatin protein 1 binds to methylated H3K9 in regions of heterochromatin.

Histone modifications are dynamic in that they are actively added and removed by histone-modifying enzymes in a site-specific manner, which is essential for coordinated transcriptional control. Each site and degree of modification is regulated by distinct enzyme families. For example, the KDM4/JMJD2 family has been shown to demethylate H3K9me3, H3K36me3, and H1.4K26me3, and the important histone acetyl transferases, ie, cAMP response element binding protein, p300, and P300/CBP-associated factor, have specific acetylation targets.

## Noncoding RNAs

Other important epigenetic players are the nonprotein-coding RNAs (ncRNAs). Recent genome-wide studies (the ENCODE project<sup>26,27</sup>) have shown that less than 2% of the human genome codes for proteins, but the genome is pervasively transcribed and produces many thousands of regulatory ncRNAs, including small ncRNAs (about 20 nucleotides in length), such as microRNAs (miRNAs), small interfering RNAs (siRNAs), P-element-induced wimpy testis-interacting RNAs (piRNAs), and various classes of long ncRNAs (>200 nucleotides in length). The most extensively studied group contains miRNAs. The number of miRNAs identified exceeds one thousand in humans. miRNAs degrade or disrupt translation of mRNA transcripts post-transcriptionally in a sequence-specific way. There are groups of miRNAs harboring the same seed sequence, and in most but not all cases, can target the same gene product. On the other hand, miRNAs are promiscuous, with one single miRNA being able to target several hundred mRNAs, although usually one particular mRNA is targeted by one miRNA.<sup>28</sup> Generation of miRNAs involves transcription to the primary miRNA that is cleaved in the nucleus by a complex containing the enzyme Drosha. This precursor miRNA is transported by the Exportin-5 pathway to the cytoplasm, where it is cleaved into a miRNA duplex (about 20 nucleotides long) by the enzyme Dicer. The mature miRNAs are loaded into the Argonaute protein of the RNA-induced silencing complex and miRNAs bind to the 3' untranslated regions of target mRNAs, translation of which will be silenced or the mRNA will be degraded. However, there is also

evidence that miRNAs can upregulate translation under certain conditions.<sup>29</sup> miRNAs and also long ncRNAs are involved in directing chromatin-modifying complexes to their site of action, indicating an important interaction with the epigenome, which can control neural plasticity.<sup>30</sup> Furthermore, there is extensive evidence for anomalies, especially in the expression profile of miRNAs in neurodegenerative disorders, particularly AD.<sup>28,31–33</sup>

Long ncRNAs (200–100,000 nucleotides in length) are a heterogeneous group forming the bulk of the human noncoding transcriptome. Various regulatory functions are assigned to them.<sup>34</sup> Among the long ncRNAs are large intergenic ncRNAs and natural antisense RNAs. Many roles have been ascribed to these molecules, including sequestration of RNA and protein molecules, thereby inhibiting their function, and, most interestingly, recruitment and guidance of epigenetic regulatory factors to their sites in chromatin.<sup>35</sup> The central nervous system contains a very large assortment of long ncRNAs, and evidence is emerging to suggest that these RNAs have an important role during development and in the adult central nervous system, including in neuronal plasticity. For example, brain-derived neurotrophic factor (BDNF) antisense RNA suppresses the function of BDNF in part by binding BDNF mRNA and in part by inhibiting transcription via recruitment of the polycomb repressor complex to the *BDNF* gene promoter region.<sup>36</sup>

## Aging

Aging is the most important risk factor for AD, and epigenetic changes have been observed in aging tissues. The importance of environmentally induced epigenetic changes was suggested by monozygotic twin studies showing that DNA methylation patterns in twins are concordant in early life, but gradually diverge during the lifespan.<sup>37</sup> There is also experimental evidence that environmental factors, even transient ones in early life, can induce AD-like pathogenesis in association with aging.<sup>38</sup> With improved technology, more extensive information is now available on the epigenetic changes associated with aging. These have indicated differences in DNA methylation depending on brain region and aging. In the most comprehensive study to date, DNA methylation was examined in >27,000 CpG sites from donors ranging in age from 0.4 to 102 years, and a strong relationship was found between DNA methylation and aging.<sup>39</sup> In the human frontal cortex, temporal cortex, pons, and cerebellum, more than 1,000 associations were found between DNA methylation

at CpG sites and age, and some of the associations were significant in all four regions. The majority of the association sites were in CpG islands, and the pattern was similar in the frontal cortex, temporal cortex, and pons, but different in the cerebellum. The correlation was positive at methylation sites showing a significant association with age in all four brain regions. The positive association between DNA methylation and age was the major trend in the majority of all the significantly associated loci in each brain region. Of the age-associated sites within CpG islands, the correlation between DNA methylation and chronological age was positive at more than 98% of sites, but was positive for only 76% of associated sites outside the CpG islands. The classes of genes at age-associated sites included DNA-binding factors and transcription factors. Given the functional nature of these classes, it is conceivable that altered epigenetic regulation at these loci may give rise to quite broad changes in transcriptional potential during the aging process. It is of interest that there are relatively few mRNA changes showing a linear association with age, although there is a tendency for gene expression to show higher variance as organisms age,<sup>40</sup> suggesting that an age-dependent increase in DNA methylation may be important for maintaining consistent gene expression with age.<sup>39</sup>

One of the characteristic impairments seen with aging is compromised memory. Preclinical studies show that epigenetic mechanisms are involved in formation and maintenance of memory. Inhibition of DNA methylation has a deleterious effect on neuronal plasticity,<sup>11</sup> which is also regulated by histone modifications. Peleg et al<sup>41</sup> observed that associative learning was impaired in 16-month-old mice compared with 3-month-old mice. This was associated with a specific reduction in acetylation of H4K12. Freeze conditioning resulted in altered expression of more than 2,000 genes in the brains of 3-month-old mice. In contrast, the hippocampal transcriptome remained almost unaltered in 16-month-old animals. Further studies indicated that the severe lack of gene expression in the 16-month-old mice is linked, at least, in part to deregulated acetylation of H4K12, which is associated with impaired transcriptional elongation of upregulated genes. It is particularly significant that the age-related memory impairment could be rescued by correcting histone acetylation.

Age-related memory loss starts in the dentate gyrus.<sup>42</sup> A recent gene expression study in the human dentate gyrus post mortem, normalized for gene expression in the entorhinal cortex that is not affected by aging, identified

17 genes showing reliable age-related changes.<sup>43</sup> The most significant change was an age-related decline in retinoblastoma-binding protein p48 (RbAp48), a histone-binding protein that is present in protein complexes involved in histone acetylation and chromatin assembly. A similar age-related decrease in expression of RbAp48 was observed in mice. A gene knockin of a dominant negative form of RbAp48 elicited memory deficits in young mice similar to those observed in older mice. Functional magnetic resonance imaging showed that dysfunction was selective for the dentate gyrus and this corresponded to a selective reduction of acetylation of histones that are known to have a relationship with formation of memory, such as H4K12<sup>41</sup> and H2BK20, a target of CREB-binding protein (CBP).<sup>44</sup> Increasing RbAp48 in the dentate gyrus of aged mice significantly increased their performance on memory tasks to a level comparable with that of young wild-type mice. Their improved memory was accompanied by increased levels of acetylated H4K12 and H2BK20. RbAp48 also interacts with CBP, a histone acetyl transferase critically involved in consolidation of memory.<sup>45</sup> The histone acetyl transferase activity of CBP was reduced in the dentate gyrus after inhibition of RbAp48. Selective reduction of CBP histone acetyl transferase activity in the dentate gyrus was also observed in aged wild-type mice, which was rescued together with the memory deficit upon upregulation of RbAp48. This exciting study showed that the dentate gyrus is a relatively selective target in normal aging, and understanding the underlying molecular mechanisms of this may help to identify treatment for aging-related memory impairment.

A recent study using mouse models demonstrated that the hypothalamus is important in systemic aging and lifespan control.<sup>46</sup> The mechanism involves an interaction between immune and neuroendocrine factors and is mediated by an aging-related increase in nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) signaling from microglial cells. This results in increased expression of immune signaling molecules, including tumor necrosis factor-alpha which, upon release, activates the inhibitor of NF- $\kappa$ B kinase subunit  $\beta$  (IKK- $\beta$ )-NF- $\kappa$ B pathway in neurons. This signaling negatively regulates the expression of gonadotrophin-releasing hormone via induction of Fos, Jun, and protein kinase C. Aging is known to be associated with downregulation of gonadotrophin-releasing hormone. Treatment of old animals with gonadotrophin-releasing hormone was found to restore new cell formation in the hypothalamus and dentate gyrus and to reverse aging-associated physiological

and histological changes as well as cognitive decline. Thus, the hypothalamus can integrate NF- $\kappa$ B-directed immunity and the gonadotrophin-releasing hormone-driven neuroendocrine system to program development of aging. Zhang et al<sup>46</sup> speculate that the IKK- $\beta$ -NF- $\kappa$ B pathway might become activated during the aging process by an epigenetic mechanism. The NAD-dependent protein deacetylases, sirtuins (SIRT6), are implicated in regulation of the lifespan in a number of organisms. It has been shown that deletion of *Sirt6* in mice leads to a markedly shortened lifespan and a premature aging-like phenotype.<sup>47</sup> The NF- $\kappa$ B family of transcription factors modulates aging-related pathways in mammals.<sup>48</sup> There are five NF- $\kappa$ B family members, each of which contains a Rel-homology DNA binding domain. In unstimulated cells, NF- $\kappa$ B is sequestered in the cytoplasm by inhibitory I $\kappa$ B proteins. Upon stimulation by diverse cell stressors (such as tumor necrosis factor-alpha, a cytotoxic cytokine), IKK- $\beta$  kinase becomes activated and phosphorylates I $\kappa$ Bs, leading to its degradation and allowing NF- $\kappa$ B to translocate into the nucleus and activate target genes. NF- $\kappa$ B induces expression of I $\kappa$ B, thus mediating inactivation of the signal.<sup>49</sup> Kawahara et al found that SIRT6 binds to the NF- $\kappa$ B subunit, RELA, and attenuates signaling by deacetylating H3K9 at target gene promoters.<sup>50</sup> It is proposed that hyperactive NF- $\kappa$ B signaling contributes to development of aging, whereas attenuation of NF- $\kappa$ B signaling by deacetylation of H3K9 at the chromatin by SIRT6 mitigates aging-associated physiological changes.

## Epigenetic alterations in AD

### DNA methylation

Studies of epigenetic changes in AD are only now starting to emerge. Hitherto, most studies analyzed DNA methylation in the brains of AD cases. In one of these studies, DNA methylation at the 5' promoter regions of a few candidate genes was investigated.<sup>51</sup> These genes were selected on the basis of hypotheses concerning the molecular mechanisms of AD, and included *microtubule-associated protein tau*, *APP*, and *presenilin-1* genes in the frontal cortex and hippocampus of both controls and AD cases at different Braak stages. No significant difference in CpG methylation was observed between the control and pathological samples. Siegmund et al<sup>52</sup> studied the methylation of 5'-CpG islands at 50 loci related to growth and development of the central nervous system. AD-associated changes were detected in two loci (SORBS3 and S100A2, encoding a cell adhesion molecule and a calcium-binding protein, respectively) that primarily reflected acceleration of age-related changes.

Wang et al<sup>53</sup> analyzed 12 potential AD susceptibility loci and did not find significant differences compared with controls. They observed interindividual variance in DNA methylation, which was particularly marked in the presenilin-1, apolipoprotein E, methylenetetrahydrofolate reductase, and DNMT1 promoters. They noted epigenetic drift (a departure from normal methylation) that was more pronounced in the AD samples than in controls. It has been reported that methylation of the binding site for transcription activator specificity protein 1 increases in the tau promoter, while that of the GC-rich repressor decreases in the human brain with age.<sup>54</sup> Tohgi et al<sup>55</sup> also reported hypomethylation of APP in the promoter region of a >70-year-old human brain, which would be consistent with an increase in APP expression with aging. However, in later studies, no difference was found in methylation of selected regions of the *APP* gene in various stages of AD progression compared with controls.<sup>51</sup> Further, the methylation status of the APP promoter region does not indicate involvement in regulation of this gene in AD.<sup>56</sup>

A study by Bakulski et al<sup>57</sup> was the first to provide a semi-unbiased, quantitative, genome-wide localization of DNA epigenetic differences in the human frontal cortex between AD cases and controls. Quantitative DNA methylation was determined at 27,578 CpG sites spanning 14,475 genes. The findings in controls indicated that the methylation state is markedly affected by age, with about the same number of sites being hypermethylated as hypomethylated with age. Compared with controls, 6% of genes featured on the array were differentially methylated in the AD samples, but the mean difference was relatively modest (2.9%). Gene ontology analysis revealed a relationship between the main disease-specific methylation loci and several molecular functions and biological processes, including hypermethylation of genes involved in transcription and DNA replication, while membrane transporters were hypomethylated. With the exception of the *PS1* gene, the methylation status of the AD candidate genes did not differ from that in controls. The CpG site with a minimum false discovery rate was located in the promoter of the *TMEM59* gene and it was 7.3% hypomethylated in the AD samples. This protein is involved in post-translational glycosylation of APP, and leads to retention of APP in the Golgi apparatus.<sup>58</sup> Methylation of this gene, especially in AD samples, decreased with age. In this careful study, an additional set of matched-pair samples, including some cases that were younger than in the original set, were analyzed with pyrosequencing, and the relationship between methylation status and AD was not found to be significant.

Further studies on *TMEM59* mRNA and protein expression also failed to show differences between AD and controls. Therefore, this genome-wide study showed rather modest changes in methylation state in the brains of AD cases versus controls. The technical limitations of this study should be borne in mind, namely that CpG islands within promoters were overrepresented in the array used and that methylation at these sites was much lower compared with that in the intragenic CpG sites (about 3% versus 75%, respectively) that may correlate better with transcription than the promoter CpG islands.<sup>59</sup> Further, as in most epigenetic studies of the human brain, the samples represented a mixed population of cells, although the critical changes may be cell type-specific. Further studies with improved techniques, including genome-wide analysis of DNA methylation and hydroxymethylation in samples enriched in neurons and specific neuron types, are required to assess the impact of DNA methylation in the pathogenesis of AD.

Sanchez-Mut et al<sup>60</sup> studied DNA methylation in the brain using a microarray containing the 5' regulatory region of 384 genes that are associated with neuroplasticity and with mental illness. In the frontal cortex of transgenic AD mice, they observed differential methylation in four genes compared with controls. Three of these genes, ie, thromboxane A2 receptor, sorbin and SH3 domain containing 3, and spectrin beta 4, were also found to be hypermethylated in the frontal cortex of human AD cases. They established that hypermethylation was associated with a reduction in the corresponding transcripts and proteins. These hypermethylation targets indicate that the CREB activation pathway and the axon initial segment could contribute to the disease. The authors point out the limitations of this study, in particular that only sequences in the microarray and 5' regions were interrogated.

It seems therefore that genome-wide analysis of DNA methylation has failed to identify marked alterations in the AD brain. In contrast, Mastroeni et al<sup>61,62</sup> used immunocytochemistry to detect methylcytosine-containing structures and reported a very severe reduction of this epigenetic mark in the AD entorhinal cortex. These studies have been extended recently and a similar, although less severe, reduction in immunocytochemically detected DNA methylation in the AD hippocampus was reported.<sup>63</sup> In contrast, Coppieters et al reported an increase in the global level of both 5-mC and 5-hmC in the middle frontal and temporal gyrus of AD cases compared with controls.<sup>64</sup>

In view of the likely influence of environmental factors in sporadic AD, the observations of Basha et al<sup>65</sup> and Wu et al<sup>38</sup>

are particularly relevant. They documented in experimental animals that an adverse environmental influence during a sensitive developmental period can elicit epigenetic changes in gene expression characteristic of the features of AD after a delay that persists until old age, including increased expression of APP and beta-site APP-cleaving enzyme 1 (BACE1) and an elevated amyloid load associated with a decrease in DNMT activity. These authors suggest that epigenetic imprinting as a result of environmental influences in early life is important. In a recent extension of these studies, they confirmed that the A $\beta$  level in the brain of 23-year-old Cynomolgus monkeys exposed to lead in infancy was elevated compared with controls.<sup>10</sup> Of 588 genes with neurobiological relevance, 22 showed altered expression (16 repressed and six upregulated). The controls showed age-dependent decreases in DNMT1 and DNMT3a, and decreased MeCP2 protein levels, whereas histone acetylation marks and H3K4me2 levels were increased. Level of all these marks were markedly suppressed in the brains of the lead-treated animals compared with those of the controls.

## Histone modifications

Histone acetylation was also investigated in mouse models of AD. In *APP/presenilin-1* double mutant transgenic mice, associative learning was impaired and this was linked with a marked reduction in H4K14 histone acetylation, while the basal level of acetylation was similar to that in controls.<sup>66</sup> A more recent study investigated the influence of A $\beta$  on histone homeostasis.<sup>67</sup> Exposure of cortical/hippocampal cultures to A $\beta$  oligomers resulted in increased levels of acetylated H3K14 and a loss of dendritic spines, which was prevented by inhibition of histone acetyl transferase. In young preplaque AD transgenic mice, markedly increased levels of H3K14ac and H3K9me2 were observed compared with wild-type nontransgenic controls. Most importantly, similar changes were observed in histone transcription activating and repressive marks in the occipital cortex of AD patients at autopsy. The other end of the AD pathology state was investigated in transgenic animals by Govindarajan et al.<sup>68</sup> They studied histone acetylation changes in a very aggressive model of AD (APPPS1-21). In these animals, pathological changes were already manifest by 2 months of age and the studies were performed at the age of 15 months, when the pathology was very advanced. Associative learning was studied after freeze conditioning, and severe impairment was found, and was associated with reduced levels of various acetylated histones in the hippocampus, but only some in the cortex.

These observations strongly support the view that AD is a disease with an epigenetic motif.

Proteolytic processing of APP by BACE1 at the  $\beta$ -site is essential for generation of A $\beta$ , which is widely presumed to be a major pathogenetic factor in AD. There have been several reports of the activity, protein, and transcript levels of BACE1 being increased in AD (for reviews see Stockley and O'Neill<sup>69</sup> and Sun et al<sup>70</sup>). Preclinical studies indicate that epigenetic mechanisms are involved in the increased expression of BACE1. Reduction of folate and vitamin B12 levels in the culture medium caused a reduction in S-adenosylmethionine levels, and the reduced methylation potential resulted in an increase in expression of BACE1 and presenilin-1.<sup>71</sup> BACE1 mRNA levels were found to be increased in the brains of old 3 $\times$ Tg-AD mice and this was accompanied by an increase in acetylated H3 at the promoter region of the gene.<sup>72</sup>

## Chromatin modification

In a recent study, changes in the organization of chromatin were detected in hippocampal neurons from AD cases.<sup>73</sup> This work explored the mechanism of tau-induced neurodegeneration. AD is also a tauopathy, with one of its characteristic features being deposition of neurofibrillary tangles composed of tau. Initially, tau-induced degeneration was examined in *Drosophila* neurons, and loss of heterochromatin was observed. The same change was also observed in hippocampal neurons from AD cases. Relaxation of heterochromatin was associated with expression of genes that were heterochromatically silenced in the controls, with genes associated with developmental processes being overrepresented. They suggest that there is a major shift in gene expression in AD toward a more fetal state. Further, their observations in *Drosophila* suggested that in tauopathies, oxidative stress-induced DNA damage upstream of heterochromatin loss triggers a sequence of cellular events leading to aberrant cell cycle activation and subsequent neuronal apoptosis. Finally, they found that changes in heterochromatin present in the AD brain are also present in peripheral blood mononuclear cells, which might provide a helpful biomarker for the disease.

## Noncoding RNAs

### MicroRNAs

Accumulating evidence suggests that alteration in the miRNA network could contribute to AD risk, and several misregulated miRNAs are implicated in the regulation of key genes involved in AD, including *APP*, *BACE1*, and *microtubule-associated protein tau*. Misregulated miRNAs can also exert an effect on the pathogenesis of AD indirectly by influencing

adult neurogenesis and the immune system. There are excellent reviews on this subject,<sup>32,33</sup> and the interaction between epigenetic mechanisms and miRNAs has also been recently reviewed.<sup>74</sup> Here, only a brief summary is given of some of the important findings.

Two important proteins in the pathogenesis of AD, ie, APP and BACE1, are controlled by miRNAs. The link with tau is strongly suggested by observations concerning conditional DICER deficiency.<sup>75</sup> The miRNAs involved in APP expression include miR-101, miR-106a, miR-520, miR-124, and let-7, and miR-298, miR-328, miR-107, miR-124, and miR-29a/b-1 are among those affecting BACE1.<sup>28</sup>

The expression of APP can be affected by miRNAs at different levels of generation of the protein, including splicing and translation. Human neurons express the short APP isoform APP695 that lacks the Kunitz-type protease inhibitor encoded by exon 7 and the OX2 domain encoded by exon 8. Longer isoforms comprising these domains are increased in AD and are associated with elevated A $\beta$  production. Both miR-124 and polypyrimidine tract binding protein 1 can alter the splicing APP. miR-124 is reduced in AD and thus may contribute to increased generation of A $\beta$ .<sup>76</sup>

Some of the miRNAs influencing translation of APP are downregulated in the AD brain, but they often also affect other functions that contribute to the pathology. For example, miR-101 is involved not only in the regulation of APP expression, but also in the inflammatory response through upregulation of cyclooxygenase-2.<sup>77</sup> The downregulation of miR-106b may also be a protective mechanism against entry into the cell cycle, since this miRNA controls two important regulators of the cell cycle, ie, retinoblastoma 1 and p21.<sup>78</sup> Other targets include p62, which is required for autophagic degradation of polyubiquitinated substances.<sup>79</sup>

miRNAs usually bind to sites on the 3'-UTR of mRNAs, although binding to the core region has also been observed. Single nucleotide polymorphism has been detected in the 3'-UTR of some of the mRNAs relevant to AD. These usually create novel miRNA binding sites and might contribute to phenotypic differences between individuals. Bioinformatic analysis identified a number of miRNA binding regions near to three polymorphic sites in the 3'-UTR of APP mRNA. Delay et al<sup>80</sup> showed that the T117C variant inhibits miR-147 binding, thereby increasing expression of APP and A $\beta$ , whereas the A454G variant increases miR-20 binding and reduces their expression. Therefore, APP 3'-UTR polymorphisms could affect the risk of AD via modulation of APP expression.

BACE1 is the rate-limiting enzyme in A $\beta$  production. Translation of the mRNA is regulated by both miRNAs and long ncRNAs (see below). BACE1 expression is reduced in vitro by miR-29a, miR-29b-1, and miR-9.<sup>81</sup> In a subgroup of patients with sporadic AD, BACE1 protein levels are increased, and this is associated with downregulation of the miR-29 cluster. Levels of miR-107 are reduced in Braak III stage patients with increased expression of BACE1 protein.<sup>82</sup> miR-298 and miR-328 bind specific sites in the 3'-UTR of BACE1 and downregulate translation of BACE1. In the transgenic AD mouse, there is a decrease in levels of these miRNAs with age, and this correlates with an increase in BACE1 levels in the brain.<sup>83</sup>

Regulation of miRNA expression by epigenetic mechanisms and reciprocal modulation of the epigenetic makeup by miRNAs is convincingly demonstrated by the effect of miR-137 on the fate of adult neural stem cells.<sup>84</sup> Overexpression of miR-137 promotes proliferation of adult neural stem cells, whereas its repression promotes differentiation. MeCP2 in coregulation with SOX2 (SRY [sex determining region Y]-box 9) inhibits expression of miR-137, resulting in promotion of adult neural stem cell differentiation. On the other hand, miR-137 represses translation of enhancer of zeste homolog 2, a polycomb group protein, causing a global decrease in the repressive mark, H3K27me3. Another example of the crosstalk between epigenetic mechanisms and miRNAs involves miR-148a, which is downregulated in AD. There is evidence from non-neuronal cells that hypermethylation of the miR-148 promoter represses its expression, whereas overexpression of miR-148 represses expression of DNMT1.<sup>85</sup>

An example of the indirect effects of miRNAs on the pathology of AD is the influence of miRNAs, including miR-137, on the metabolism of ceramide.<sup>86</sup> Ceramide levels are increased in AD, and the rate-limiting enzyme in the de novo pathway, ie, serine palmitoyl transferase, is under the control of miR-137. The increased ceramide levels may augment production of A $\beta$  by recruiting BACE1 and  $\gamma$ -secretase to lipid rafts.

Cognitive impairment is one of the major consequences of AD. The hippocampus is a key brain structure for formation of memory and is affected early in AD. Using parallel sequencing, Zovollis et al<sup>87</sup> assessed the complete mouse hippocampal miRNAome. They observed that some miRNAs are highly enriched in the hippocampus, including miR-34c, which was significantly upregulated in 24-month-old mice and in AD transgenic animals. They observed that this miRNA exerts a negative effect on associative learning.



Levels of miR-34c are elevated in the hippocampi of AD patients, suggesting that this miRNA could be involved in the cognitive impairment associated with AD and could be a suitable target for therapy.

## Long noncoding RNAs

Changes in the expression of long ncRNAs have also been noted in neurodegenerative diseases, including AD. BACE1 antisense RNA is highly expressed in the AD brain. In contrast with most antisense RNA functions, it stabilizes BACE1 mRNA and so promotes generation of A $\beta$ .<sup>88</sup> The mechanism involves the competition of this long ncRNA with miR-485-5p that reduces BACE1 expression.<sup>89</sup> Both ncRNAs bind to the same sequence in exon 6 of the BACE1 transcript, and BACE1-AS stabilizes gene expression by successfully competing with miR-48-5p for the binding site.

The inflammation associated with many neurodegenerative diseases, including AD, induces expression of the 17a long ncRNA, which controls the alternative splicing of the gamma-aminobutyric acid (GABA)<sub>B</sub> receptor 2 (GABA<sub>B</sub>R2), reducing both formation of the canonical form and GABA<sub>B</sub> signaling. Expression of 17a long ncRNA in neuroblastoma cells enhances the secretion of A $\beta$  and the A $\beta$ <sub>42</sub> to A $\beta$ <sub>40</sub> ratio.<sup>90</sup>

Parenti et al<sup>91</sup> found that more than 200 genes are differentially expressed in rat cortical neurons after exposure to A $\beta$ . One of these is an antisense transcript to Ras-related protein associated with diabetes (RAD18), an enzyme that is involved in the DNA repair mechanism. The RAD18 antisense transcript exerts post-transcriptional control over RAD18, and has been suggested to contribute to susceptibility to apoptosis. There is intensive research activity under way to obtain more knowledge about noncoding RNAs and their functions, which will provide important new information about neurological diseases, including AD.

## Environmental factors

Although the heritability of AD is high (about 60%),<sup>92</sup> the influence of environmental risk factors is still significant. This has been clearly indicated by studies in monozygotic twins, which show divergence with age in susceptibility to disease and this is associated with remarkable differences in epigenetic marks affecting the gene expression profile.<sup>37</sup> Epidemiological evidence indicates that there are many risk factors for AD, such as high blood pressure, cardiovascular problems, and diabetes.<sup>93</sup> The role of environmental factors in interaction with the genetic background has recently been reviewed, primarily considering transgenic mouse models of AD.<sup>94</sup>

Here, only one of the environmental factors is considered, ie, B vitamin deficiency, that occurs frequently in elderly people and is considered to be a risk factor for both cardiovascular disease and dementia, including AD,<sup>95</sup> although this claim is still controversial.<sup>95,96</sup> Elevated serum homocysteine levels are usually associated with B vitamin deficiency. Homocysteine is an intermediate in one-carbon metabolism. It is converted to S-adenosylhomocysteine, which is a powerful inhibitor of methylation reactions. Among the inhibited reactions is methylation of the catalytic subunit of the protein phosphatase PPA2, which is essential for formation of the catalytically competent enzyme, which is an important tau dephosphorylase and thus may contribute to formation of neurofibrillary tangles (see Vafai and Stock<sup>97</sup>). Importantly, DNA methylation can also be inhibited by S-adenosylhomocysteine and this has important consequences for gene expression, including genes critically involved in AD.

There are three vitamins that play an important role in the one-carbon metabolism: the remethylation of homocysteine to methionine requires folate and B12, and in the trans-sulfuration pathway, homocysteine is converted to cystathionine in a reaction involving vitamin B6. Deficiency in these vitamins can lead to hyperhomocysteinemia and a decrease in the S-adenosylmethionine/S-adenosylhomocysteine ratio, causing impaired methyltransferase function.

Although still controversial, B vitamin deficiency and elevated homocysteine may be risk factors for dementia.<sup>95,96,98</sup> In a recent small-scale clinical trial in elderly patients with mild cognitive impairment, it was found that brain atrophy could be slowed with homocysteine-lowering B vitamins and this was associated with a slowing of both the cognitive and clinical decline.<sup>99</sup> This is relevant, given that over half of those over 70 years of age with mild cognitive impairment develop AD.

There are preclinical observations showing convincingly that vitamin B deficiency leading to elevated homocysteine levels results in gene expression changes that are relevant to the pathogenesis of AD. It was found that expression of *presenilin-1* and *BACE1* genes is increased and that the *PS1* gene promoter is hypomethylated in animal models of AD.<sup>100</sup> In these experimental animals, the production of A $\beta$  was increased and cognitive impairment in AD transgenic animals was registered even before the stage of intense plaque deposition.

## Treatment possibilities

Until now, clinical trials on AD using different treatments have shown no or limited beneficial effects. The most recent

Phase III trials using passive anti-amyloid immunotherapy were also disappointing, although a subsequent analysis showed a significant effect on cognition in patients with mild AD.<sup>101</sup> Currently, only acetylcholinesterase inhibitors and the N-methyl-D-aspartate antagonist memantine are approved for the treatment of AD, but their effect is rather limited. However, as a result of the problems with the clinical trials, it is now realized that treatment must be started early, because the pathogenesis predates the manifestation of clinical symptoms by decades, and must continue for a relatively long time. In addition, important biomarkers are now available for monitoring disease progression. The Alzheimer's Prevention Initiative, a 5-year trial funded by government and private sources, was announced last year and will test the efficacy of an A $\beta$  antibody in asymptomatic members of an extended Colombian family who carry a *PS-1* mutation that causes early-onset AD.<sup>102</sup>

## Epigenetic treatments

In comparison with the more classical attempts at treatment, epigenetic treatment may offer potential benefits.<sup>103</sup> The effect of histone deacetylase (HDAC) inhibitors has been tested in preclinical studies done in animal models of AD. There are four classes of HDACs: I, II, and IV are Zn-dependent enzymes comprising eleven members; class III [the SIRT6s] are different, in that they are NAD<sup>+</sup>-dependent deacetylases that also possess mono-ADP-ribosyltransferase activity, and will be considered separately.

## Zinc-dependent histone deacetylases

Class I HDACs containing HDAC 1, 2, 3, and 8 are primarily localized in the nucleus, where they regulate histone acetylation. Class II HDACs shuttle between the cytoplasm and the nucleus and this movement is regulated via calcium-calmodulin-dependent protein kinase-mediated phosphorylation. All class IIa HDACs (4, 5, 7, and 9) have relatively low deacetylase activity and most likely act in concert with other HDACs. Class IIb HDACs include HDAC6 and HDAC10. HDAC6 is the main cytoplasmic deacetylase that targets  $\alpha$ -tubulin, among other cytoplasmic proteins. There is only one member in class IV, ie, HDAC 11, that has characteristics of both HDAC class I and class II. HDAC1 and HDAC2 have a very similar structure and are sometimes present in the same nuclear complexes. However, their functions are completely different. HDAC2 is the major negative regulator of synaptic plasticity processes,<sup>104</sup> whereas HDAC1 exerts a powerful neuroprotective function.<sup>105</sup> HDAC3 is also involved in cognitive control, but its role is more refined

than that of HDAC2.<sup>103</sup> The important transcription factor, CREB, is activated by phosphorylation on S133. PP1 is the enzyme that dephosphorylates and inactivates CREB, and is targeted to the transcription factor by interaction with HDAC8.<sup>106</sup> HDAC5 is a regulator of response to stress and addiction.<sup>107</sup> This enzyme plays an important role in models of mood disorders, which is significant given that affective disorders are a risk factor for AD.<sup>107</sup>

In animal models of AD, treatment with HDAC inhibitors resulted in markedly improved cognitive function<sup>66,68,108–110</sup> (for a recent review see Fischer<sup>111</sup>). Histone acetylation is severely reduced in AD transgenic animals. Treatment with HDAC inhibitors restores histone acetylation in the hippocampus, but not in the cerebral cortex.<sup>68</sup> Rescue of learning and memory function by HDAC inhibitors is accompanied by increased expression of synaptic plasticity-associated functions, such as glutamate receptors and various synaptic proteins.<sup>68,109</sup> Particularly important is that the treatment with an HDAC inhibitor at a stage of advanced AD-type pathology is still effective.<sup>68,109</sup> Further, it is promising that, even after induction of neuronal degeneration following conditional activation of cyclin-dependent kinase 5, when experimental animals had forgotten a learnt task, treatment with HDAC inhibitors rescued memory, indicating that memories were not lost, only compromised.<sup>112</sup> In addition, environmental enrichment had the same effect as HDAC inhibitors. Environmental enrichment is one of the means by which cognitive impairment can be reversed in old dogs showing neuropathological alterations similar to those seen in aging humans, including accumulation of A $\beta$  in the brain.<sup>113</sup>

These preclinical studies are very promising in view of possible treatments for AD, especially considering that valproic acid has been used since the 1960s in neurological/psychiatric disorders and functions in part as an HDAC inhibitor. Further, the therapeutic use of HDAC inhibitors has been approved in certain types of cancer.<sup>114</sup> Nevertheless, there are important problems that must be overcome before these inhibitors can be used in the treatment of AD. The first cohorts of HDAC inhibitors have been wide-spectrum drugs affecting many HDAC species. Although new developments produced a few relatively selective HDAC inhibitors, such as MS-275 for HDAC1<sup>115</sup>, ACY-775 for HDAC6<sup>116</sup> and PCI-34051 for HDAC8<sup>117</sup> the problem is that HDACs are components of multiprotein complexes that modify chromatin structure and it is not yet clear how well the properties of the drugs observed in isolated enzymes translate in the function of these complexes.<sup>118</sup> Further, HDACs are lysine deacetylases and have many targets besides the histones, so their application may have unwanted side effects. This is an

important menace especially after long-term treatment essential in a neurodegenerative disorder. The better understanding of the roles of the distinct HDAC proteins in the adult brain is therefore critical for the development of selective drugs for AD. An important approach involves genetic manipulation of the HDACs in preclinical studies. This has also been applied to the CNS, including by the groups of Tsai and Fischer.<sup>104,119</sup> A specific challenge for the application of HDAC inhibitors in neurodegenerative diseases is the penetration of the drugs through the blood–brain barrier. In spite of these problems, it is encouraging that experience with cancer patients indicates few serious toxic effects after the use of HDAC inhibitors.

## Sirtuins

The SIRT family in mammals has seven members, which differ in their subcellular localization and some lack deacetylase activity. SIRT1 is the most extensively studied member and is mainly localized in the nucleus, but also has some cytoplasmic functions. SIRT2 is predominantly cytoplasmic and deacetylates tubulin, but may also shuttle to the nucleus. SIRT3, SIRT4, and SIRT5 are mitochondrial enzymes, whereas SIRT6 is associated with chromatin and SIRT7 is in the nucleoli.

Since the observation in 1999 that SIRT2 prolongs life in yeast,<sup>120</sup> the SIRTs have been linked to the regulation of aging and the influence of caloric restriction on the lifespan in many species, although there is controversy about the effect of SIRTs on the lifespan.<sup>121</sup> Nevertheless, there is no doubt that SIRTs positively affect quality of life, being potent protectors against aging-associated pathologies, such as diabetes, cardiovascular disease, and neurodegeneration. The effect of SIRTs has been studied in animal models of some of the devastating neurodegenerative diseases, such as AD, Huntington's disease, and Parkinson's disease, and there is an excellent review of this research.<sup>122</sup>

Resveratrol has been used to activate SIRTs in many studies. This is a polyphenol found mainly in grapes and red wine, and has been reported to mimic caloric restriction via its effect on SIRTs. There is some controversy about the effect of this compound, which definitely has some other effects as well, including antioxidant activity. A recent study suggests that resveratrol activates SIRT1 via an allosteric mechanism, and only substrates that have specific hydrophobic motifs in the binding pocket, such as peroxisome proliferator-activated receptor- $\gamma$  coactivator 1 $\alpha$  and Forkhead box O3a, facilitate activation.<sup>123</sup>

Activation of SIRT1 ameliorated amyloid pathology in model systems of AD. One of the suggested mechanisms involves activation of nonamyloidogenic processing of APP via  $\alpha$ -secretase. This is mediated by SIRT1 deacetylation and

thus activation of the retinoic acid receptor  $\beta$ , a transcription activator of ADAM10 that functions as an  $\alpha$ -secretase.<sup>124</sup> The nonamyloidogenic pathway is also activated by SIRT1 via inhibition of expression of Rho-associated, coiled-coil containing protein kinase, and thus disinhibits  $\alpha$ -secretase.<sup>125</sup>

SIRT1 is implicated in the effect of caloric restriction on the lifespan. There is epidemiological evidence that reduced caloric intake improves cognitive performance in elderly people and reduces the risk of AD in apolipoprotein E4 carriers.<sup>126,127</sup> One of the epigenetic mechanisms for adaptation to restricted energy intake involves the function of SIRT1. In eukaryotic cells, the most energy-consuming process is ribosome biosynthesis, which adapts to changes in intracellular energy status. The mechanisms that links energy status to ribosome biosynthesis involve SIRT1 function. The mammalian ribosomal DNA locus is under the epigenetic control of protein complexes, such as the energy-dependent nucleolar silencing complex, involving SIRT1 and the methyltransferase SUV39H1. A reduction in energy status can activate SIRT1 via an increase in the NAD<sup>+</sup>/NADH ratio, leading to deacetylation of histones and SUV39H1, which is thereby activated, causing repressive methylation of H3K9 and thus mediating the epigenetic silencing of ribosomal gene expression.<sup>128,129</sup>

Activation and inflammation of microglia are part of the neuropathological picture in neurodegenerative conditions, including AD. SIRTs can mitigate this pathology. SIRT1 interacts with and deacetylates the NF- $\kappa$ B subunit, RelA/p65, and thus inhibits NF- $\kappa$ B-induced expression of genes involved in inflammation.<sup>130</sup> SIRT6 is also involved in attenuation of NF- $\kappa$ B signaling via deacetylation of H3K9 at appropriate sites on chromatin.<sup>50</sup> Because of the promising observations regarding the beneficial effect of resveratrol-activated SIRT1 in preclinical studies, three clinical trials are now under way to test the effects in patients.

Overexpression or drug-induced activation of SIRT1 was found to be protective in animal models of AD and other neurodegenerative disorders (see Herskovits and Guarente<sup>122</sup>). It should be noted that the different members of the SIRT family may have different influences. For example, SIRT1 and SIRT2 have opposing effects in models of neurodegeneration in Parkinson's disease in vivo, indicating that target specificity within this class of HDACs is important.

## Noncoding RNAs

Noncoding RNAs have some definite therapeutic potential, for example RNA-based drugs mimicking primarily small interfering RNAs have been developed for many diseases.<sup>131</sup> The first clinical trial of miRNA-based therapy is currently in

progress in patients with chronic hepatitis C.<sup>132</sup> However, the blood–brain barrier poses a special challenge for delivery of such substances to treat neurodegenerative diseases. Finally, it is important to recognize and avoid adverse effects.<sup>133</sup>

## Lifestyle

The heritability of AD is high.<sup>92</sup> Nevertheless, factors not genetically associated with AD and environmental influences play a significant role in AD. Epidemiological studies indicate that vascular factors, hypertension, metabolic syndrome, diabetes, depression, and nutrition, are important risk factors in AD.<sup>134</sup> In addition, the incidence of dementia is related to social, cognitive, and physical activity.

Lifestyle plays an important role in the prevention/delay of onset of AD. Although most investigations have focused on animal models of AD, there are human studies that lend support to the preclinical observations. Physical exercise has been investigated for its impact on risk of development of the disease (for a review, see Intlekofer and Cotman<sup>135</sup>). A meta-analysis of reliable studies has recently been conducted, and its results indicate that physical exercise elicits improvement in cognitive function in patients with dementia or mild cognitive impairment and in healthy individuals. One year of exercise in seniors improved not only spatial memory, but also attenuated loss of gray matter, increased hippocampal volume, and improved connectivity in the brain in comparison with sedentary individuals.<sup>136</sup> Another meta-analysis of nearly 34,000 nondemented subjects who participated in prospective studies showed that nearly 10% of subjects showed cognitive decline during follow-up. Physical activity protected against such cognitive deterioration. Interestingly, the protection provided by moderate/low physical activity was nearly as good as that provided by high activity (hazard ratio 0.65 versus 0.62).<sup>137</sup> In addition to the increasingly recognized role of physical exercise in maintaining cognition, exercise also influences AD pathology. AD biomarkers, including amyloid deposition in the brain, monitored by Pittsburgh compound-B imaging, and tau, phosphorylated tau, and A $\beta$ <sub>42</sub> in the cerebrospinal fluid indicate that the progress of AD pathology is mitigated by engagement in exercise.<sup>138</sup>

Preclinical studies showed that running increases learning (ie, improved the performance of mice on a water maze test) and adult neurogenesis in the dentate gyrus.<sup>139</sup> Significantly, the aging-associated decline in learning and neurogenesis is corrected, in part, by running.<sup>140</sup> The positive effect of exercise on cognition involves neurotrophic factors, such as BDNF, insulin-like growth factor 1, and vascular endothelial growth factor. BDNF, in particular, is critical for both the developing and the mature nervous system. It

is vital for both the survival and maintenance of neurons and also plays an important role in synaptic plasticity.<sup>141,142</sup> Therefore, it is significant that BDNF levels are reduced in the AD brain,<sup>143</sup> and according to preclinical observations, BDNF expression can be upregulated as a result of physical exercise.<sup>135</sup>

The *BDNF* gene consists of eight 5'-untranslated exons and one protein-coding 3' exon.<sup>144</sup> BDNF transcripts contain one of the eight 5' exons spliced to the protein-coding exon. Because the eight untranslated exons are regulated by specific promoters that respond to different transcription factors, the expression profile for the gene is most flexible. The distribution of the various 5' exon-containing transcripts is tissue-specific; in the brain, the most abundant form is the exon IV BDNF.<sup>144</sup> Furthermore, Aid et al<sup>144</sup> demonstrated that epigenetic regulation could play a role in a promoter-specific and cell specific manner in BDNF expression. The final BDNF transcript contains only the 5' extended protein-coding exon. Translation results in the formation of a BDNF precursor peptide (proBDNF) that is functional in its own right and mediates effects that are the opposite of those induced by the mature peptide. Thus, proBDNF binding to the p75 neurotrophin receptor plays an important role in apoptosis and elicits long-term depression,<sup>145,146</sup> whereas the mature BDNF peptide is critical for long-term potentiation<sup>147</sup> and the maintenance of neurons. The proBDNF is proteolytically processed to the mature BDNF by plasmin extracellularly and proprotein convertases, including furin, intracellularly. Interestingly, the mouse and rat BDNF gene loci do not code antisense transcripts, suggesting that the mechanisms of regulation for the rodent and primate *BDNF* gene differ substantially.<sup>144</sup>

Studies on post mortem tissue from suicide victims showed that not only BDNF, but also the TrkB receptors for this neurotrophin, are decreased in the brain.<sup>148</sup> Preclinical studies identified epigenetic mechanisms involved in the regulation of *BDNF* gene expression in depression-like situations (for review see Vialou et al<sup>149</sup>). Under these conditions, there is increased methylation of BDNF promoters in the hippocampus associated with decreased BDNF expression. Chronic social defeat-induced depression-like situations lead to a transient decrease in the level of acetylated H3 in many brain regions, including the hippocampus and the nucleus accumbens. This is followed only in the latter region by a more persistent increase, which may be mediated by the repression of HDAC2 and is accompanied by sustained induction of BDNF. It is important that the increased acetylated H3 and decreased HDAC2 have also been detected in the nucleus accumbens of depressed

humans. Studies in experimental animals showed that the effect of antidepressant treatment in reversing depression-induced downregulation of BDNF in the hippocampus is mediated by increased histone acetylation at the promoter via downregulation of HDAC5.<sup>150</sup> Depression is one of the significant AD risk factors, and BDNF is also downregulated in the AD brain and a reduction is already detectable in mild cognitive impairment (for review, see Allen et al<sup>143</sup>).

During development, the brain is sensitive to environmental influences, such as parental care. Maltreatment in this period increases the risk of behavioral disorders. It has been shown that infant animals raised under adverse conditions have persistently decreased BDNF levels in different parts of the brain. Compared with controls, the regulatory region of BDNF exon IV is methylated. Drug-induced demethylation reversed the reduced gene expression. Female rodents that were maltreated as infants showed similar abusive behavior towards their offspring, and both the behavior and epigenetic changes at the BDNF promoter were transmissible.<sup>151</sup>

Also, there are “good” and “bad” mothers among rodents. The corresponding behavior and stress sensitivity show corresponding characteristics in the offspring, which are lasting and transmissible, and are mediated by epigenetic regulation of gene expression involving, among other changes, regulation of expression of glucocorticoid receptors in the hippocampus.<sup>152</sup> The consequences of adverse influences during infancy involve hyperactivity of the hypothalamic-pituitary-adrenal axis through increased expression of corticotrophin-releasing factor via reduced promoter methylation,<sup>153</sup> and of arginine vasopressin<sup>154</sup> in the paraventricular nucleus, which regulates corticotrophin release and thus elevated corticosteroid burden. The mechanism of increased arginine vasopressin production involves stress-induced activation of protein kinases that phosphorylate MeCP2, causing its release from the arginine vasopressin enhancer, thus activating expression of the gene. This increased stress reactivity is sustained into adulthood.<sup>154</sup> Further observations showed that stable modification of DNA methylation is also a feature of modulation of  $\alpha$ -estrogen receptors triggered by early life experience.<sup>155</sup> Recently, a study of the effect of mother care was extended to investigation in the offspring brain of transcriptional and epigenetic changes in rat chromosome 18, which contains the glucocorticoid receptor. Maternal care was found to have a broad effect on transcription involving hundreds of genes.<sup>156</sup> The translational relevance of these experimental studies became evident in the recent observation of increased DNA methylation of the glucocorticoid receptor gene promoter and decreased glucocorticoid receptor mRNA expression in the brains of suicide victims with a history of childhood abuse, compared

with victims with no such abuse.<sup>157</sup> In further studies, genome-wide DNA methylation was investigated in the hippocampus of abused suicide completers. In comparison with controls, more than 300 differentially methylated promoters were identified.<sup>158</sup> Genes involved in neuronal plasticity were among the most significantly differentially methylated. Another observation that environmental conditions in early life can cause persistent epigenetic changes in humans is the effect of periconceptual undernutrition during the Dutch famine towards the end of the Second World War; 60 years later, affected individuals showed reduced DNA methylation of the imprinted insulin-like growth factor-2 gene in whole blood.<sup>159</sup>

Based on the observation that early postnatal exposure to lead elicits gene expression relevant to AD pathology after a long latent period in old experimental animals, the LEARN (Latent Early-Life Associated Regulation) model was proposed for the pathogenesis of AD.<sup>160</sup> As mentioned before, recent research indicates that some epigenetic modifications are detectable in the brains of 23-year-old monkeys exposed to lead in infancy.<sup>10</sup>

Nutrition is an important lifestyle factor. Epidemiological data suggest a protective role for n-3 fatty acids, B vitamins, and antioxidant nutrients in cognitive decline and dementia.<sup>161</sup> Although studies in experimental animals have supported the positive role of most of these supplements, clinical trials have generally been negative.<sup>162</sup> The current trend in nutritional epidemiology is to analyze dietary patterns rather than individual supplements. Encouraged by the beneficial effect of the Mediterranean diet on cardiovascular disease, this diet has also been tested for its effect on cognitive function, and most studies, but not all, indicate that it reduces the risk of cognitive decline, including AD. A recent meta-analysis of a number of reliable epidemiological studies concluded that higher adherence to the Mediterranean diet is associated with a lower rate of cognitive decline and a reduced risk of AD.<sup>163</sup> However, in the same issue of the journal containing this meta-analysis, an epidemiological study of a large sample of elderly women was also published, which failed to show a positive effect of the Mediterranean diet on cognitive function, although an increased ratio of monounsaturated to saturated fatty acids was independently associated with better average cognition at older ages.<sup>164</sup> Randomized clinical trials are required to determine whether or not the Mediterranean diet is a beneficial strategy to ward off cognitive decline, including AD.<sup>162</sup> Although the primary aim in the PREDIMED (Effects of the Mediterranean diet on the primary prevention of cardiovascular diseases) study was to establish an association between the Mediterranean diet and cardiovascular disease,<sup>165</sup> the secondary outcome of the trial

was the effect of this diet on neurodegenerative disorders, and the results should be available soon.

In animal experiments, caloric restriction, environmental enrichment, and physical activity paradigms play a part in lifestyle interventions. Caloric restriction ameliorated cognitive deficit in various transgenic AD animals and reduced the amyloid load in the brains of, eg, 3×Tg-AD mice<sup>166</sup> and Tg2576 transgenic mice.<sup>125</sup> As mentioned previously, this effect involves a SIRT1 activity-mediated increase in  $\alpha$ -secretase function.<sup>124,125</sup> Most of the observations in transgenic mouse models of AD show that an enriched environment has a positive effect on cognitive function.<sup>167</sup> The associated epigenetic mechanisms have been studied in the p25/Cdk5 model of neurodegeneration.<sup>112</sup> Environmental enrichment restored apparently lost memory even after neurodegeneration had occurred, and the effect was similar to that of HDAC inhibitors. Environmental enrichment has many effects, including an increase in BDNF expression in the brain and increased adult neurogenesis. Running is considered an element of environmental enrichment. Kobilic et al<sup>168</sup> found that exercise is the critical factor in mediating the effect of environmental enrichment on increased BDNF levels and neurogenesis in the adult hippocampus. Further, it was found that exercise can transform a subthreshold learning event into long-term memory.<sup>169</sup> This depends on upregulation of hippocampal BDNF and involves the association of acetylated H3K8 with BDNF promoters. The effect of exercise is comparable with that of HDAC inhibitors, suggesting that exercise engages epigenetic mechanisms. Upregulation of BDNF is critically involved in the positive effect of exercise on neuronal plasticity. Gomez-Pinilla et al<sup>170</sup> observed that exercise stimulates BDNF promoter demethylation, increases H3 acetylation, and reduces the level of HDAC5, which has been implicated in regulation of the *BDNF* gene.<sup>150</sup> Exercise activates cAMP response element binding protein, so can affect expression of genes involved in neuronal plasticity, including *BDNF*. As mentioned earlier, the influence of physical activity on AD pathology and cognitive function has been studied in transgenic AD mice (for a review, see Chouliaras et al<sup>94</sup>), and it is quite plausible that epigenetic mechanisms are involved in the positive effects.

## Conclusion

An estimated 24 million people had dementia worldwide in 2011, the majority thought to have AD,<sup>171</sup> with a great increase in numbers anticipated worldwide.<sup>4</sup> Currently, there are no effective treatments for the disease, and the clinical trials primarily based on the amyloid hypothesis of the disease have not been successful. However, important lessons were learnt from these trials, and it is expected that the corrected study designs will

produce meaningful results. The new approach involves prospective long-term trials started in humans when they are clinically normal, but have the genetic defect that will lead ultimately to the disease.<sup>102</sup> In the meantime, it is encouraging that epidemiological studies indicate the importance of lifestyle factors in the prevention/delay of disease onset. Preclinical studies indicate that many lifestyle factors impact relevant genetic expression via epigenetic mechanisms. Further, in animal models of AD, treatments that affect the epigenome, in particular modulating the histone code, have a positive effect on certain aspects of AD pathology, and most importantly on cognitive function. These studies have provided evidence for HDAC inhibitors exerting a positive influence similar to that of lifestyle factors. Although some of the experimental drugs of interest are already in use in the treatment of cancer, there are important hurdles that must be overcome before their application can be considered in neurodegenerative diseases. Almost all the available inhibitors are broad-spectrum agents and have limited permeability through the blood–brain barrier. More species-specific inhibitors are needed, but in the meantime the existing agents may facilitate identification of brain targets for specific medication. Further, ncRNAs, especially miRNAs, have significant therapeutic potentials, but more basic research is required before they can be considered for treatment. Overall, it seems that targeting epigenetic mechanisms is a promising avenue for future treatment of many neurodegenerative diseases, including AD.

## Disclosure

The author reports no conflicts of interest in this work.

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