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Short running title: Epigenetic targets for Alzheimer's disease

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- **Abstract:**

Alzheimer's disease is a progressive and fatal neurodegenerative disease affecting 50 million people worldwide, characterized by memory loss and neuronal degeneration. Current treatments have limited efficacy and there is no cure. Alzheimer's is likely caused by a combination of factors, providing several potential therapeutic targets. One area of interest is the epigenetic regulation of gene expression within the brain. Epigenetic marks, including DNA methylation and histone modifications, show consistent changes with age and in those with Alzheimer's. Some epigenetic regulation has been linked to disease pathology and progression and are the focus of current research. Epigenetic regulators might make promising therapeutic targets yet challenges need to be overcome to generate an efficacious drug lacking deleterious side-effects.

- **Keywords:** Chromatin; epigenetics; Alzheimer's; dementia; methylation; acetylation; ageing; histone; non-coding RNA

- **Main body of text**

Introduction

Alzheimer's disease (AD), the most common form of dementia, is characterized by progressive memory loss and cognitive decline and was predicted to cost the world economy $1 trillion in 2018 [1]. Pathologically, extracellular Amyloid Beta (Aβ) plaques and intracellular neurofibrillary tangles (NFT) composed of hyperphosphorylated tau (p-tau) aggregates are seen within the cortex and hippocampus. These pathologies are thought to be the result of abnormal proteolytic processing of amyloid precursor protein (APP), a pathway termed the amyloid cascade hypothesis [2]. Consequent synaptic and neuronal loss is thought to underlie the cognitive deficits seen [3, 4]. Currently there is no cure; treatments targeting toxic Aβ plaques and cholinergic neuronal loss have had limited efficacy and provide only mild symptomatic relief. These treatments however are only employed at late stages in the disease and their lack of efficacy suggests earlier pathologies, such as epigenetic modifications, contribute to the onset and progression of dementia and uncovering such mechanisms would greatly help therapeutic developments. Mutations in genes such as *APP*, presenilin-1 (*PSEN1*) and presenilin-2 (*PSEN2*) can be

attributed as the cause for familial AD, however in regards to sporadic AD which accounts for 90% of AD cases, monozygotic twin studies show discordance for AD suggesting alternative mechanisms contribute to sporadic disease pathology [5]. Genome Wide Association Studies (GWAS) have identified >20 loci including single nucleotide polymorphisms in Apolipoprotein E (*APOE*) and Microtubule Associated Protein Tau (*MAPT*) genes which are associated with increased risk of late onset sporadic AD [6]. However these have a weak effect size, highlighting the contribution of other pathways, in the pathogenesis of the disease. Alternative hypotheses include neuroinflammation, reduced glucose metabolism, epigenetic regulation, mitochondrial dysfunction, and reduced vasculature in the brain [7-9], although it is likely AD is a consequence of a multitude of genetic and environmental factors.

The term epigenetics, introduced by Conrad Waddington in 1942, describes molecular pathways which combine to modulate our gene expression into a particular phenotype without invoking changes to the underlying DNA sequence [10]. Epigenetic hallmarks include DNA methylation, post translational modifications of histones (acetylation and methylation) and the influence of non-coding RNA. As we age our epigenome undergoes changes which is manifest as a drift toward hypermethylation at specific DNA locations but a widespread, global DNA hypomethylation [11]. It appears in AD, in which age is the biggest risk factor, the levels of localised hypermethylation and global hypomethylation are more pronounced compared to age matched controls [12, 13]. These data suggest that changes to our epigenome start to occur at an earlier age in individuals with AD or develop more rapidly, thus implicating altered epigenetic mechanisms as a potential key pathology in AD. The epigenetic modifications to our DNA and chromatin are catalyzed by a plethora of enzymes and non-coding RNA transcripts. These enzymes present as potential druggable targets for the treatment of AD whereby their altered activity could be modified therapeutically to reverse or prevent some of the epigenetic changes associated with AD.

Changes in DNA methylation at key genes is linked to Alzheimer's disease

Methylation of DNA (Figure 1A) was the first epigenetic mark identified and has been by far the most studied. This has been facilitated by technology that allows precise identification and quantification of methylation, the robust nature of DNA and the relative ease of its isolation from tissue. Overall there is good evidence from many groups that patterns of DNA methylation are altered in individuals with AD: these changes are summarised as increased methylation at specific genes associated with AD but an

overall reduction in methylation globally [12, 13]. Decreased methylation may be a consequence of altered one carbon metabolism and the heavy use of available S-Adenosyl methionine (SAM) to combat increased reactive oxygen species (ROS). Increased methylation at specific genes is presumably derived from the recruitment of individual DNA methyltransferase (DNMT) enzymes to specific genomic regions [14]. Age is the biggest risk factor for AD and it is clear that DNA methylation changes (local hypermethylation and global hypomethylation) seen with ageing are similar to those observed in patients with AD [15, 16].

DNA methylation is deposited by the action of DNA methyltransferase (DNMT) enzymes (Figure 1A), of which there are 3 in humans (DNMT1, DNMT3a and DNMT3b). Increased methylation at the *DNMT1* gene has been associated with AD [13] suggesting globally, DNMT1 levels might be reduced. Aged mice show reduced levels of *DNMT3* in the hippocampus. Expression of ectopic *DNMT3a* using viral delivery enhanced cognitive performance in object recognition and spatial memory tasks, whilst shRNA knockdown of *DNMT3a* in young mice impaired cognitive functioning [17]. Inhibitors of DNMT enzymes exist, and are currently used in treating certain cancers, though they lack specificity for individual enzymes and from the available functional data, widespread DNMT inhibition is likely to have a negative effect on cognition. Increased methylation of a number of other genes within the genome of patients with AD has been observed. These include the genes; *PSEN1*, *APOE*, Methylenetetrahydrofolate reductase (*MTHFR*) and *DNMT1* [13]. DNA methylation would be expected to result in reduced expression and of these genes which is thought to predispose an individual to AD. However much of the data is collected from post-mortem brain so it is unclear if the altered methylation levels contribute to or are caused by AD.

Overall the genome in patients with AD shows a general hypomethylation which might reflect altered one carbon metabolism. However there are some specific regions which show hypomethylation suggesting there is also a more targeted mechanism involved. Like the targeted hypermethylation, this hypomethylation is seen at genes that have been associated with AD and has the potential to contribute to AD pathology. These hypomethylated regions include intron 3 of the Down Syndrome cell adhesion molecule-like 1 (*DSCAML1*) gene, 5 tau kinase encoding genes (*MAPK10, MARK3, CAMK2A, CAMK2B and DYRK1A*) and 2 genes (*CDK5R2* and *CDK5RAP2*) that activate CDK5 and are associated with increased tau phosphorylation [18]. The hypomethylated enhancer at the *DSCAML1* region may actually regulate expression of the Beta-secretase 1 (*BACE1)* gene: it was associated with the *BACE1* gene in 3D space and

hypomethylation of this region correlated with increased *BCAE1* mRNA at early stages of AD [18]. As well as differences in the average level of methylation, a recent study that examined stability and variability in DNA methylation found that variable methylation in AD was concentrated around regions that are involved in regulating the expression of genes associated with neurogenesis and development [19]. Thus, levels of methylation at specific regions are potentially important, as is the reduced stability of methylation at enhancers; which increases the propensity for methylation levels to change in response to signals. Both mechanisms appears to contribute to disease progression.

Given how long we have known about and studied DNA methylation it is perhaps surprising how little we still understand about its involvement in complex diseases such as AD. Indeed although there is now general acceptance of the idea that DNA methylation is reversible there is no incontrovertible evidence, nor a unified consensus, on the mechanism(s) responsible for DNA demethylation. Overall targeting DNA methylation is potentially the most challenging given that; we don't understand how the DNA methylation changes contribute to AD, there are no inhibitors or activators available that are specific for individual DNMTs and the apparent involvement of increases in DNA methylation in AD at some genes but decreases in DNA methylation at others.

Deregulation of histone acetylation in Alzheimer's disease negatively impacts cognitive functioning and neuronal survival

Post translational modifications of histone proteins are important epigenetic events, implicated in a range of physiological and pathological processes. Acetylation, of lysine residues within histones (Figure 1B) promotes an open chromatin state, more amenable for transcription. Acetylation then is associated with increased gene expression and its deposition catalysed by histone acetyltransferases (HATs, Figure 1B), whilst histone deacetylases (HDACs, Figure 1B) catalyse deacetylation to remove acetyl groups and repress gene expression [20].

There is evidence that levels of HDACs are altered in AD, specifically both HDAC2 and HDAC6 protein levels are elevated in the cortex and hippocampus of post mortem brain tissue from patients with AD compared to controls [21-23]. Contrastingly, a recent PET study using [^{14}C] Martinostat, a radioligand that binds class 1 HDACs, reported a reduction in global HDAC levels in AD affected regions such as the posterior cingulate

cortex and hippocampus [24]. In fact reduced HDAC levels correlated with mild cognitive impairment suggesting altered HDAC regulation may be an early event in the pathogenesis of AD [24]. While these studies appear contradictory [^{14}C] Martinostat does not distinguish between the Class I HDACs (HDAC1, 2, 3 and 8) so while levels of HDAC2 might go up, levels of other HDAC s may go down. An overall decrease in HDAC levels would be predicted to result in a rise in histone acetylation levels, and there is some suggestion that increased levels of histone acetylation are associated with AD [25]. In a H3K27ac association study, widespread variation in acetylation, of genes associated with AD, was seen in entorhinal cortex samples from AD patients compared to matched controls, with hyperacetylation seen at regions near *PSEN1*, *PSEN2* and *MAPT* genes and hypoacetylation seen near *APP* gene. Variations in H3K27ac levels were also identified to overlap with AD GWAS regions [26]. These data suggest that altered acetylation is linked to AD though whether it contributes to or is a result of AD is unclear. H3K27 is also a site of methylation and as both types of modification cannot exist on the same lysine together, changes in acetylation could well be the result of an altered level of H3K27 methylation. To determine if there is any overlap in the regions that show altered DNA methylation and histone acetylation, Marzi et al. [26] compared the genomic regions that showed changes in each modification. There was little direct overlap between regions showing altered H3K27Ac levels and regions showing altered DNA methylation [26] suggesting the existence of multiple, possibly independent mechanisms, though regions of altered DNA methylation did show enrichment for altered acetylation perhaps indicating that DNA methylation changes do have a downstream influence on acetylation.

Increased HDAC2 levels seen in patients with Alzheimer's disease would suggest it may be a target for Alzheimer's therapy and support for HDAC2 as a potential therapeutic target is provided by evidence from a number of animal models. In the CK-p25 mouse model of neurodegeneration, HDAC2 expression in the brain is increased and corresponds to repression of genes involved in neuroplasticity [27]. Mice showed poor performance in the Morris water maze and a fear condition test but this was improved to levels similar to wild type animals with siRNA knockdown of HDAC2, suggesting that the increased level of HDAC2 was the cause of the deficit [27]. Genetic overexpression of HDAC2, but not HDAC1, specifically within mouse brain neurones, results in decreased dendritic spine and synapse density within hippocampal slices and correlates with reduced memory formation [28]. Mice in which the *Hdac2* gene had been knocked out showed an opposite phenotype: they had an enhanced response to associative learning tasks, showed enhanced LTP in the hippocampus and an improved spatial memory [28, 29]

though their episodic memory is not different to wild type animals [29]. HDAC3 may also make a contribution to cognition. Knockout of HDAC3 from neurones in the hippocampus resulted in enhanced object recognition memory though spatial memory differences were not tested [30]. In contrast, knockout of *Hdac1* in forebrain neurones had no clear effect on memory or hippocampal LTP [29]. In fact, reduction of HDAC1 may be deleterious. In the CK-p25 mouse model, the p25 protein, which is abnormally regulated in AD, was shown to interact with and inhibit the function of HDAC1 [31]. Inhibition of HDAC1 in this way was shown to be neurotoxic through promoting DNA damage and neuronal death. Increasing HDAC1 could prevent this damage. Together these data suggest that increased HDAC2 and HDAC3 activity has a negative impact on cognition while reduced HDAC1 activity may be neurotoxic, consistent with the observation that inhibition of HDAC enzymatic activity with SAHA can improve cognition but does not prevent neurodegeneration. An effective therapeutic agent would therefore need to inhibit HDAC2 and/or HDAC3 but not inhibit HDAC1.

As a potential therapeutic avenue, the use of HDAC inhibitors has shown some promise in animal studies and HDAC inhibitors appear to reduce AD pathology *in vitro* and memory impairments *in vivo*. The HDAC inhibitor M344 reduced Aβ accumulation, tau phosphorylation, and lowered BACE and APOE4 gene expression in HEK cells overexpressing the APP variant containing the Swedish mutation (KM670/671NL, [32]). *In vivo*, treatment of 3xTg AD mice (*APP* Swedish, *MAPT* P301L, and *PSEN1* M146V mutations) with M344 enhanced spatial memory and novel object recognition [32]. Oral administration of MS-275 in APP/PS1 transgenic mice reduced Aβ deposition [33] whilst W2 improved performance in the Morris water maze and decreased Aβ levels in 3xTg AD mice [34]. Valproic acid, Sodium Butyrate and SAHA (Figure 1B) all enhanced the response to fear conditioning in mutant APP/PS1 mice when compared to vehicle treated littermates [35]. Treatment of 3xTg AD mice with RGFP-966, increased histone acetylation and correlated with reduced Aβ accumulation, tau phosphorylation and increased performance in spatial and memory and object recognition tests [36]. RGFP-966 shows some selectivity for HDAC3 (IC_{50} of 1.45μM) but is only 3.1 and 4.9 fold more selective toward HDAC3 than HDAC1 and 2 respectively [36] and it is not clear which HDAC(s) is the important target for these physiological responses.

Inhibition of HDAC2 and/or HDAC3 but not HDAC1 would be the goal for the development of a HDAC based therapy. The catalytic domains of HDAC1 and HDAC2 are extremely similar, both in amino acid sequence and 3D structure. The deacetylase domain is 366 amino acids and an alignment of these

domains from the human HDAC1 and HDAC2 proteins shows that they are 94% identical (Figure 2A). Crystal structures of both HDAC1 and 2 catalytic domains have been determined [37, 38] as part of complexes with partner proteins and overlaying of these domains shows that the 2 structures occupy identical configurations (Figure 2B). From these data it does not seem that it would be possible to generate small molecules that are able to bind to the enzyme pocket of HDAC2 to inhibit its activity but be excluded from the pocket of HDAC1. One possibility would be to incorporate other parts of HDAC2 into the drug design, for example the region around the entrance to the active site and produce a molecule that could interact with HDAC2 to block access to the active site. Molecules such as antibodies or aptamers may be able to achieve such a goal though their physiochemical properties make them less than ideal as drugs. The development of subtype specific HDAC inhibitors is a current focus, however faces many challenges which are discussed here [39]. An alternative strategy to targeting specific HDAC's, is to inhibit other components of the complex they are part of and that are required for proper function. A functional screening for co-regulators of HDAC2 revealed the transcription factor Sp3 works in conjunction with HDAC2 to regulate transcription. Knockdown of Sp3 using shRNA in CK-p25 mouse model of AD reduced HDAC2 recruitment to the promoter of genes in primary neuronal cultures which was accompanied by an increase in histone acetylation of several synaptic genes [23]. Disruption of the HDAC2-Sp3 interaction by expression of a C-terminus segment of HDAC2 rescued memory deficits in fear conditioning paradigms in the CK-p25 model. Like HDAC2, Sp3 is elevated in the hippocampus of AD patients [23] and thus development of a small molecule capable of inhibiting this interaction should reduce HDAC2 function without also the HDAC1 activity that is important for cell survival.

Alternative ways to restore the balance of histone acetylation would be to increase acetyltransferase. Whilst increased levels of HDAC are associated with AD pathology, reduced levels of the HAT enzyme, CREB-binding protein (CBP), is an early event in APPswe/PSEN1dE9 mice and accompanied by reduced histone acetylation levels in the hippocampus [40]. CBP activity is promoted through increased cAMP and cGMP levels and one way to increase cGMP is to inhibit phosphodiesterase activity which is responsible for cGMP turnover. A combination of the HDAC inhibitor voronistat and the phosphodiesterase-5 (PDE5) inhibitor tadalafil enhanced LTP in hippocampal slices from APP/PS1 mice and improved spatial and associative memory performance in Tg275 mice (Swedish APP KM670/671NL mutation) [41] as did CM-144, a molecule that combines HDAC inhibition activity with inhibition of PDE5 [42]. Direct activation of

CBP using CSP-TTK21 (Figure 1B), delivered through intraperitoneal injection, was also able to increase acetylation of H2B and restored plasticity in the hippocampus and spatial memory [43].

Targeting histone acetylation does seem to have promise as a strategy to alleviate the effects of AD. Identifying a selective inhibitor of HDAC2 and/or 3 that does not inhibit HDAC1 appears quite challenging though the ability to activate HATs either directly or through modulation of second messengers does provide alternative ways to meet the same objectives.

Two Histone methylation marks show altered patterns of deposition in AD

A post-translation modification of histones that has been the focus of much interest is methylation. Both lysine and arginine residues within histone N-terminal tails can be modified by methylation by up to 2 (Arginine) or 3 (Lysine) methyl groups (for a review of histone methylation and the enzymes that add and remove methyl groups, see [44]). While there are a number of residues that can be methylated, two specific examples of histone methylation have been associated with Alzheimer's disease, H3K9me2 and H3K4me3.

H3K9me2

Dimethylation of H3K9 is controlled through a histone methyltransferase complex containing both histone methyltransferases EHMT1 (also known as GLP) and EHMT2 (also known as G9a, Figure 1C) [45]. An active complex *in vivo*, appears to require the presence of both enzymes [46]. In humans, levels of EHMT1 but not EHMT2 mRNA were found to be higher in the prefontal cortex of post-mortem brain tissue from individuals with AD compared to aged matched controls. If both enzymes are required for a functional complex, the outcome resulting from the change in expression of one enzyme but not the other is not easily predictable without a precise handle on stoichiometry. In the Alzheimer's mouse models Tg2576 and FAD (mice with *App* K670N/M671L+I716V+V717 and *Psen1* M146L+L286V mutations) there are increased global levels of H3K9me2 in the prefontal cortex while levels in hippocampus but not in the striatum of FAD mice are also higher [47, 48]. In FAD mice there is a specific increase of H3K9me2 at 717 genes including the *Gria2* and *Grin2B* genes which encode the GluR2 AMPAR and the NMDA2B receptor subunits respectively. These genes show reduced expression levels as evidenced by reduced AMPA and NMDA currents [48]. Functionally, FAD mice show reduced performance in spatial memory and object recognition behavioural tests but this is improved with inhibition of EHMT1/2 by either BIX01294 or

UNC0642 (Figure 1C), [48]. Neither BIX01294 nor UNC0642 treatment resulted in a reduction of Aβ plaques or Tau phosphorylation though, giving further support to the idea that there is a not a requirement to remove Aβ plaques or reduce Tau phosphorylation in order to improve cognition in patients with AD [49].

On the face of it the above data would suggest that inhibition of EHMT1/2 would be a useful strategy in AD treatment. The availability of inhibitors precludes the requirement for a large scale screening programme and, unlike HDAC inhibitors, the available inhibitors do show selectivity. Indeed it has been shown that peripheral administration of BIX01294 in mouse is able to reduce levels of H3K9me2 in the cortex and enhance gene expression as evidenced by increased *Bdnf* mRNA levels [50]. However, reducing EHMT1/2 activity too much may result in alternative problems. In humans a haploinsufficiency of EHMT1 is the cause of Kleefstra syndrome, a disorder characterised by intellectual disability. This is mimicked in mouse models where deficiency of either *Ehmt1* or *Ehmt2* results in similar learning deficits [51]. Reduced EHMT1/2 levels in the nucleus accumbens (NAc) are also associated with addiction and the anatomical and behavioural response to repeated exposure to cocaine [52]. After a series of cocaine administrations, mice show a preference for cocaine in a conditioned place preference test and anatomically have an increased density of dendritic splines on neurones in the NAc. Ectopic expression of EHMT2 is able to prevent the changes in spine density and alteration in behaviour, while inhibition of EHMT1/2 or reduction of EHMT2 levels augmented the behavioural preference for cocaine and reduced the levels of H3K9me2 in the NAc [52]. These data are consistent with a model whereby high levels of EHMT1/2 activity might reduce strength and/or plasticity of synapses whereas low levels would promote stronger synaptic connections. Thus in AD, high levels of EHMT1/2 in the cortex result in reduced synapse strength and negatively impacts cognition while in response to repeated cocaine exposure, reduced EHMT1/2 levels in the NAc results in enhanced synapse strength of neurons within the reward pathway and promotes addiction.

The EHMT1/2 complex has been proposed to act as an epigenetic regulator involved in synaptic scaling, maintaining firing rates of neurones within a physiological range [53]. A major hypothesis of cognitive deficits is that they arise from the inability of neurones to respond to environmental changes and an imbalance in chromatin modifying proteins that underpin transcriptional plasticity will reduce neuronal dynamics and have a negative effect on cognition. Thus it would seem likely that there is a “sweet spot”

of EHMT1/2 activity where either too little or too much leads to cognitive deficiencies. Given that increased EHMT1/2 is seen in the prefontal cortex of patients with AD, then partial inhibition may be able to restore functional levels of activity. However the EHMT1/2 levels do not appear to be increased globally, for example, they are not increased in the striatum while levels in other areas have not been examined [48]. It is not clear what the levels of EHMT1/2 are in the NAc but reduction of EHMT1/2 activity in this area below normal levels would clearly not be welcome because it would risk promoting addictive behaviour. It seems that we should first address whether altered EHMT1/2 levels are associated with AD and once we are in possession of that information we can determine if EHMT1/2 inhibition has potential to treat symptoms of AD without generating substantial side effects.

H3K4me

In contrast to H3K9me2, tri-methylation of H3K4 is generally associated with transcriptional activation. A number of mutations that affect genes which regulate H3K4 methylation are associated with neurodevelopmental disorders that involve intellectual disability (reviewed in [54]) though disruption of these genes has not been associated with neurodegeneration in humans. Levels of H3K4me have been seen to be higher at some genes and lower at others in the CK-p25 neurodegenerative mouse model [55], which shows neuronal loss, cognitive decline, Tau phosphorylation and neurofibrillary tangles similar to AD but has no build-up of Aβ plaques [56]. H3K4me3 is deposited by the KMT2A (Figure 1D) and KMT2B histone methyltransferases. In the CK-p25 mouse, *Kmt2a* levels are reduced compared to wild type, mice but *Kmt2b* levels are not. Fifty two percent of the genes showing reduced H3K4me3 in the CK-p25 mouse also show reduced H3K4me3 in the *Kmt2a* knockout mouse [57]. These data suggest that, at least a proportion of the H3K9me3 reduction in the CK-p25 mouse is due to reduced *Kmt2a* levels. The functional significance of *Kmt2a* reduction in the CK-p25 mice is supported by the observation that knockdown of *Kmt2a* or *Kmt2b* in the prefontal cortex resulted in deficiencies in spatial memory as measured by the T-arm maze and radial maze tests [58, 59]. Neither neurodegeneration nor neurofibrillary tangles however have been reported in mice lacking *Kmt2a* perhaps indicating that these responses to CK-p25 overexpression are a result of a mechanism independent from H3K4 methylation.

Kmt2a and *Kmt2b* show quite distinct changes when knocked down suggesting they have quite distinct functions [59]. As they both catalyse the same reaction, specificity for histones in particular genomic areas

is most likely brought about by their interaction with individual protein complexes. This is significant for drug development as any small molecule that can regulate KMT2A, is likely to also regulate KMT2B. To compensate for reduced KMT2A levels we would want to potentiate KMT2A activity though this may be challenging to do with a small chemical and an alternative strategy would be to inhibit the enzymes responsible for removing the H3K4 methylation. A potential additional issue is that increased H3K4me3 appears to be associated with genes expressed in microglia [55] and may contribute to the inflammatory response. Thus increasing global H3K4me3 levels could result in increased microglia activity and enhanced microglia activity has been suggested to contribute to the pathogenesis of AD [60].

A number on non-coding RNAs are attractive therapeutic targets, influencing cognition and amyloid pathology

Non-coding RNA (ncRNA) are functional RNA molecules which do not code for proteins. They function as epigenetic regulators of gene expression by their involvements in transcriptional and post transcriptional gene silencing, a direct involvement in heterochromatin formation, and interactions with protein complexes involved in DNA methylation and histone modifications [61-63]. Categorisation of ncRNAs is based on their size; short ncRNA's (including micro RNA's (miRNA) of 19-24 nucleotides, small interfering RNA (siRNA) of 20-25 nucleotides and piwi-interacting RNA (piRNA) of 26-31 nucleotides) and Long ncRNAs (lncRNA, 200 nucleotides or more).

Several studies have characterised altered expression levels of specific ncRNAs with AD in humans. In a microarray analysis of AD brains compared to age-matched controls, 24 lncRNAS were found to be upregulated and 84 downregulated [63]. The miR-29 family of miRNA's were reported to be reduced globally in brains of AD patients brains [64] while other miRNA's including miR-7, miR-27a and miR-206 were increased in the temporal cortex [65, 66]. Animal models of AD also demonstrate ncRNA dysregulation; the Tg2567 mouse model, like human AD brains, shows increased miR-206 levels as well as a number of other miRs and injection of the antagomir to miR-206 (AM206) into the third ventricle improved the performance of the mice in conditional and spatial memory tests, possibly through the increase in BDNF levels that it induced [65]. Induction of AD in rats by the injection of oligomeric Aβ1-42 into the ventricles resulted in upregulation of 93 and the down regulation of 90 miRNAs [67]. All of these

data identify an alteration in ncRNA expression after AD has developed and there have been no reported mutations in any ncRNA associated with AD in GWAS studies. Together these observations point toward a role for ncRNA that might contribute to the phenotype and pathogenesis of AD but are not directly responsible for the initiation of AD. However given the ability to design specific interacting partners to bind to ncRNAs and robust methods which easily quantify very low levels of them, they may have utility as biomarkers for AD development [68] or as a way to alleviate specific symptoms such as reduced cognition [65].

Alongside neurodegeneration, cognitive decline is the symptom most closely associated with AD. A number of ncRNAs have been implicated in the regulation of cognition through the modulation of synaptic plasticity. For example the lncRNA, GM12371, is associated with transcriptionally active chromatin (Figure 1E) and knockdown of GM12371 reduces expression of a host of genes important in synaptic plasticity, lowers spine density and inhibits enhancement of synaptic transmission [69]. Levels of the lncRNA BC200 in the cortex show a 65% reduction with ageing in healthy indiviudals, however individuals with AD show an upregulation [70]. A second study did not identify increased BC200 levels in plasma from patients with AD [71], whether this indicates that plasma levels of BC200 are not indicative of levels within the brain or that increased BC200 is not a consistent event in AD is not clear. LoNA is another lncRNA whose function is associated with reduced plasticity. LoNA is found predmoniantly associated with the nucleolus within cells and thought to be inportant in rRNA expression [72]. Administration of LoNa to the hippocampus of wild type animals impaired spatial memory while mice lacking LoNA spent more time on novel object exploration. Increased expresison of LoNa is seen in brains from APP/PS1 mice, and knockdown of LoNa in these animals restored rRNA levels and improved performance in a Morris water maze to levels similar to wild type animals (Li et al., 2018). Given it is easier to knockdown ncRNAs than to enhance their expression, LoNA provides an attractive therapeutic target for congitive enhancement, whether this would also reduce neurodegeneration is still awaiting experimental data.

In addition to modulating synaptic plasticity, ncRNAs may also contibute to amyloid pathology af AD. BACE1 is a key enzyme which initates the formation of the toxic Aβ1–42 peptide from APP cleavage, and thus both *BACE1* and *APP* genes are pivatol in AD pathlogy. In AD brains, miR-29a and miR-29b-1 were decreased and this was associated with abnormally high BACE1 protein expression [64]. Bioinformatic analysis identified miR29a and 29b-1 target sites on the *BACE1* mRNA and using a luciferase reporter

construct, BACE1 was shown to be under the regulation of miR-29a and 29b-1. Furthermore, overexpression of miR-29a was sufficient to reduce Aβ peptide levels in HEK293 cells, suggesting a potential protective role of miR-29a and 29b-1 in reducing BACE1 expression to lower the Aβ burden [64]. The microRNA, miR-16 is also downregulated in brains of individuals with AD and was identified as a potent inhibitor of APP, BACE1 and Tau expression. Delivery of a synthetic miR-16 mimic into mouse brain reduced expression of BACE1, APP and Tau in the hippocampus of WT mice, suggesting a potential to reduce expression of a number of genes that promote amyloid pathology [73]. In 10 month old Tg2576 AD mice, microarray analysis identified that miR200b/c levels were upregulated in the brain. The increased levels are proposed to be a protective mechanism in response to elevated Aβ1–42 in the brain. Transfection of primary murine neurons with miR-200b/c reduced Aβ1–42 secretion into the media and infusion with miR-200b/c into the brains of mice treated with oligomeric Aβ, restored memory defects on the Barnes Maze [74].

As a therapeutic strategy, mimics of specific miRNAS appear to have potential in reducing AD progression. Additionally, miRNA mimics overcome drawbacks of conventional small molecule drugs as they can target multiple genes and affect non druggable protein targets (e.g non enzymatic proteins). However poor stability and challenges in delivery currently limit their therapeutic use; chemical modification to miRNA mimics is being investigated to reduce their vulnerability to nuclease degradation and improve stability *in vivo* while use of viral vectors can increase transduction efficacy [75]. Lastly, miRNAs need only bind to partially complementary mRNA sequences. An impact of this imperfect base pairing, means one miRNA or miRNA mimic can recognise and bind multiple mRNAs. The corollary to this is a potential for off target effects which may potentiate the disease further.

- **Future Perspective:**

Epigenetic modifications in AD appear to influence cognition though, with the exception of HDAC1 activity, there is little evidence for a pivotal involvement in neuronal degeneration. Mutations in a number of genes that modify chromatin are the cause of syndromes associated with reduced intellectual ability eg Rubinstein-Taybi syndrome resulting from mutation in the CREB binding protein (*CBP*, [76]), Rett syndrome caused by mutation in methyl-CpG binding protein 2 (*MECP2*, [77]), Kleefstra syndrome resulting from mutations in *EHMT1* [78] and Weidemann-Steiner syndrome caused by mutations in *KMT2A* [79]*. There is some evidence that links reduced HDAC1 activity to neurotoxicity [31] but when

tested, improvements in cognition in animal models are not normally associated with increased number or preservation of neurones suggesting that the neurodegeneration and cognitive decline seen in AD may occur through independent mechanisms. On the basis of the available evidence, we would hypothesise that restoring normal epigenetic function in patients with AD would improve cognition of the patient but would be unlikely to reduce or prevent the neurodegeneration. However a quality of life enhancement provided through enhanced cognition is a substantial gain to the individual.

Non-coding RNAs may prove to be more useful in combatting the amyloid pathology associated with AD and miR-16 or miR-29 could be a useful way to reduce BACE1 and APP and limit amlyoid build up. Challenges associated with non-coding RNA treatments are the ability to deliver enough RNA to the target site and provide modifications to the RNA so that the molecules have a sufficient half life to make their use in a treatment regimen feasible. Targeting BACE1 has so far proved ineffective in ameliorating cognitive deficits in clinical trials [80] but maybe a two-pronged combination therapy, one arm targeting cognition and the other neurodegeneration could be a way toward an effective treatment.

** A review of chromatin modifying implicated in cognitive disorders can be found here [81].*

Figure legends

Figure 1. Overview of the chromatin modifications reported to be altered in AD. Shown is a region of DNA wrapped around a histone octamer. **A)** DNA can be methylated at CpG dinucleotides by DNA methyltransferase (DNMT) enzymes. The DNA can be demethylated though the mechanism(s) responsible for demethylation is unclear. **B)** Lysine residues within Histone N-terminal tails can be acetylated by histone acetyltransferase (HAT) enzymes and the acetyl group can be removed by a Histone deacetylase (HDAC) enzyme. Inhibitors of HDACs, such as SAHA and the HAT activator, CSP-TTK21, have shown potential to enhance memory in mouse models of AD. **C)** Histone H3 lysine 9 (H3K9) can be di-methylated by a complex containing the methyltransferases EHMT1 and EHMT2. The methyl groups can be removed by a lysine demethylase (KDM). Both BIX01294 and UNC0642 are small molecules that can inhibit EHMT1/2 and enhance memory in a mouse model of AD. **D)** Histone H3 lysine 4 (H3K4) can be tri-methylated by lysine methyltransferase (KMT) 2a and the methyl groups removed by a lysine demethylase (KMD). **E)** The non-coding RNA, GM12371 (shown in red) is found associated with active chromatin as judged by colocalisation with histone H3 are acetylated at lysine 27 (H3K27Ac) and is important to maintain gene expression of synaptic plasticity and associated proteins.

Figure 2. HDAC1 and HDAC2 catalytic domains show a high level of sequence and structural identity. A) Alignment of the amino acid sequences encoding the catalytic domain of HDAC1 (amino acids 8-374, top) and HDAC2 (amino acids 2-368, bottom). Identical amino acids are shown in blue and differences are

highlighted in red. **B)** Two views of the superimposed catalytic domains of HDAC1 (yellow) and HDAC2 (blue) The domains were extracted from structures in the protein Data Bank [82]; PDB ID: 4BKX and PDB ID: 4LY1, superimposed using Vector Alignment Search Tool+ [83] and viewed using iCN3D [84]

- **References:** (visit our for authors page for formatting requirements and to download our endnote package)

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Figure 1

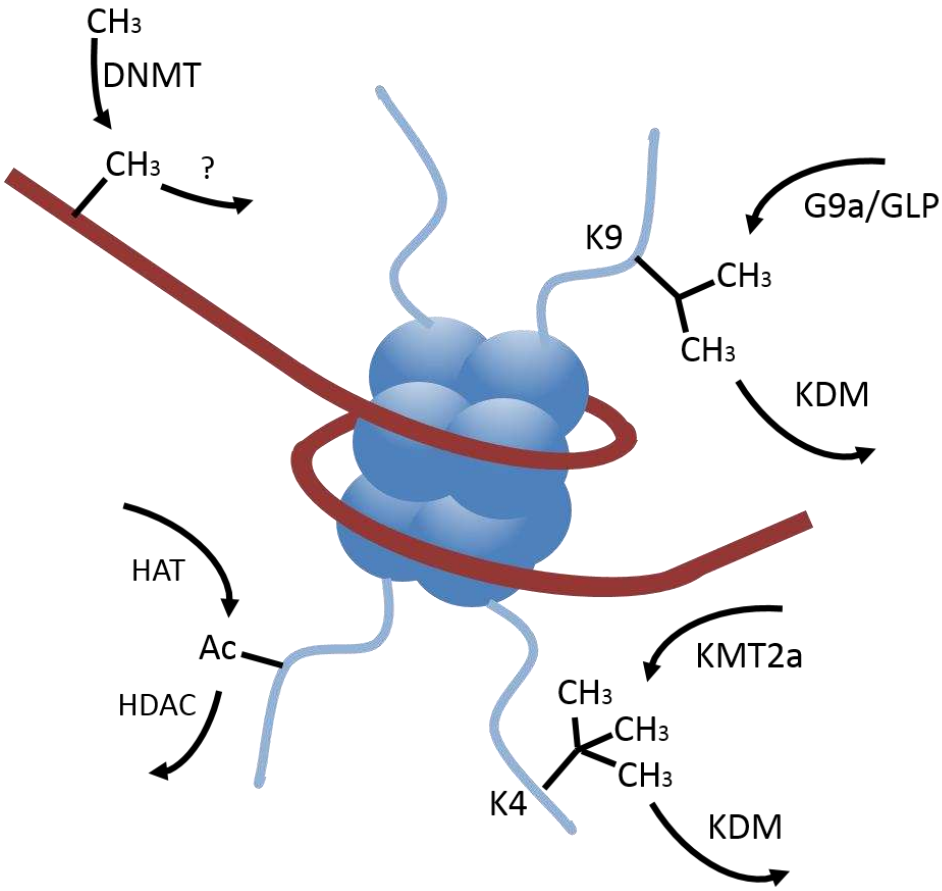
CH3
DNMT
CH3
?
G9a/GLP
K9
CH3
CH3
KDM
HAT
Ac
HDAC
KMT2a
CH3
CH3
CH3
K4
KDM

Figure 2

A)

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HDAC1   8 RRKVCYYYDGDVGNYYYGQGHPMKPHRIRMTHNLLLNYGLYRKMEIYRPHKANAEEMTKY 67
HDAC2   2 KKKVCYYYDGDIGNYYYGQGHPMKPHRIRMTHNLLLNYGLYRKMEIYRPHKATAEEMTKY 61
HDAC1  68 HSDDYIKFLRSIRPDNMSEYSKQMQRFNVGEDCPVFDGLFEFCQLSTGGSVASAVKLNKQ 127
HDAC2  62 HSDEYIKFLRSIRPDNMSEYSKQMQRFNVGEDCPVFDGLFEFCQLSTGGSVAGAVKLNRQ 121
HDAC1 128 QTDIAVNWAGGLHHAKKSEASGFCYVNDIVLAILELLKYHQRVLYIDIDIHHGDGVEEAF 187
HDAC2 122 QTDMAVNWAGGLHHAKKSEASGFCYVNDIVLAILELLKYHQRVLYIDIDIHHGDGVEEAF 181
HDAC1 188 YTTDRVMTVSFHKYGEYFPGTGDLRDIGAGKGKYYAVNYPLRDGIDDESYEAIFKPVMSK 247
HDAC2 182 YTTDRVMTVSFHKYGEYFPGTGDLRDIGAGKGKYYAVNFPMRDGIDDESYGQIFKPIISK 241
HDAC1 248 VMEMFQPSAVVLQCGSDSLSGDRLGCFNLTIKGHAKCVEFVKSFNLPMLMLGGGGYTIRN 307
HDAC2 242 VMEMYQPSAVVLQCGADSLSGDRLGCFNLTVKGHAKCVEVVKTFNLPLLMLGGGGYTIRN 301
HDAC1 308 VARCWTYETAVALDTEIPNELPYNDYFEYFGPDFKLHISPSNMTNQNTNEYLEKIKQRLF 367
HDAC2 302 VARCWTYETAVALDCEIPNELPYNDYFEYFGPDFKLHISPSNMTNQNTPEYMEKIKQRLF 361
HDAC1 368 ENLRMLP 374
HDAC2 362 ENLRMLP 368
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B)