Alzheimer's Disease and Histone Code Alterations

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Abstract

Substantial progress has been made in identifying Alzheimer's disease (AD) risk-associated variants using genome-wide association studies (GWAS). The majority of these risk variants reside in noncoding regions of the genome making their functional evaluation difficult; however, they also infer the presence of unconventional regulatory regions that may reside at these locations. We know from these studies that rare familial cases of AD account for less than 5% of all AD cases and autosomal dominant mutations in APP, PSEN1 and PSEN2 account for less than 10% of the genetic basis of these familial cases [1]. The sporadic form of AD, while more complex, still has a substantial genetic component evidenced by observational studies where 30–48% of AD patients have a first degree relative who is also affected [2]. In addition, the strongest risk factor after age is the APOE E4 polymorphism, and more than 20 other risk variants have been identified to date, reviewed in two recent papers [3, 4]. Monozygotic twin studies have revealed a discordance for AD, implicating that a combination of epigenetic and genetic factors are likely involved in the development of AD [5].

Keywords

Epigenetics • Histone modifications • Human brain

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17.1 Introduction

Substantial progress has been made in identifying Alzheimer's disease (AD) risk-associated variants using genome-wide association studies (GWAS). The majority of these risk variants reside in noncoding regions of the genome making their functional evaluation difficult; however, they also infer the presence of unconventional regulatory regions that may reside at these locations. We know from these studies that rare familial cases of AD account for less than 5% of all AD cases and autosomal dominant mutations in APP, PSEN1 and PSEN2 account for less than 10% of the genetic basis of these familial cases [1]. The sporadic form of AD, while more complex, still has a substantial genetic component evidenced by observational studies where 30–48% of AD patients have a first degree relative who is also affected [2]. In addition, the strongest risk factor after age is the APOE E4 polymorphism, and more than 20 other risk variants have been identified to date, reviewed in two recent papers [3, 4]. Monozygotic twin studies have revealed a discordance for AD, implicating that a combination of epigenetic and genetic factors are likely involved in the development of AD [5].

Epigenomic regulation encompasses DNA and histone modifications and the higher-order architecture of DNA associating with histones, alongside a plethora of transcription factors/proteins. These associations are plastic and responsive to environmental stimuli and dictate whether a specific region of DNA will be repressed, transcribed or involved in controlling the expression of other gene segments over time. In the context of Alzheimer's disease, there has been a significant shift in literature, from searching for common disease-associated variants to epigenome-wide exploration of these complex interactions, structures and modifications. Techniques for genome-wide profiling of peaks for different histone modifications, have facilitated a rapid increase in the characterisation of these modifications and the identification of specific genes they regulate across the genome in relation to specific diseases, such as AD.

This chapter will review histone modifications in the context of AD disease with a focus on studies of post-mortem human brain as well as pharmacological intervention strategies that have been tested in vivo/in clinic.

17.2 Histone Modifications

17.2.1 Enzymes That Regulate Histone Modifications

Histone modifications include the addition of methyl, acetyl, phospho and other groups to specific amino acid residues on the N-terminal tails of histones H2A, H2B, H3 and H4. The different modifications and/or combinations of modifications on a given tail determine the availability of bound DNA for transcription. Each modification is maintained by the balanced and opposing actions of enzymes: acetyltransferases add acetyl groups and deacetylases remove, methyl transferases add

methyl groups (mono-, di- or trimethyl groups can be added) and demethylases remove, kinases add phosphate groups and phosphatases remove, reviewed previously [6].

It is important to note that each enzyme group contains multiple proteins/enzymes. For example, there are at least 18 known histone acetyltransferases (HATs), more aptly renamed as lysine acetyltransferases (KATs), and the major subgroups include Gcn5 *N*-acetyltransferases (GNATs), MYST (MOZ, ybf2, Sas3, Sas2, Tip60) and p300 and CBP subfamily (p300/KAT3b, CBP/KAT3a, PCAF/KAT2a) [7]. Similarly HDACs are divided into 11 main subclasses, which can be further divided into 38 different sequence variants of the canonical sequence [8].

Early studies investigating the role of these enzymes in development have highlighted their significant roles in learning and memory. For example, mice lacking the HAT, CBP develop impaired memory function [9, 10]. Mice lacking some isoforms of HDACs such as HDAC2 and HDAC3 show improved learning [11, 12], while loss of HDAC4 and HDAC5 has been shown to impair memory function [13–15].

A recent study by Anderson and colleagues demonstrated that in comparison to mouse models of AD, where relatively high concentrations of HDAC3 and HDAC4 were observed in the brain, the same isoforms were undetectable in the human AD prefrontal cortex [16]. A previous study has shown that HDAC4 is undetectable and low levels of HDAC3 were measured in human brain [17]. Interestingly reduced HDAC4 levels in humans has been linked to mental retardation [18], emphasising the need for isoform selectivity if HDACs were to be targeted therapeutically in AD. Anderson and colleagues also showed that HDAC1 and HDAC2 were decreased and HDAC5 and HDAC6 were significantly increased in AD compared to control cases [16]. HDAC6 overexpression in AD has been observed previously [19].

It is also important to note that enzymes that add or remove different chemical groups on histone tails do not only act on histone molecules but a range of different proteins within the cell. For example, investigations of the acetylome in three different human cell lines have revealed 3600 acetylation sites across 1750 different proteins [20], while in human liver samples, another study found 1300 acetylation sites spread across 1042 proteins [21]. If all the unique proteins are combined between the two studies, then the human acetylome contains at least 2500 proteins that can be acetylated [22]. The sheer magnitude of molecules therefore regulated by HATs and HDACs raises the need for caution and highlights the need for molecular specificity of therapeutics targeting histone acetylation.

17.2.2 APP Processing and Histone Modifications

Tip60 is an acetyltransferase that interacts with the APP intracellular domain (AICD) [23] and Fe65, an adapter protein, resulting in translocation of this 'AFT' complex to the nucleus to alter gene expression [24]. The AFT complex has been shown to regulate APP itself [25], along with stathmin, a molecule involved in Tau

pathology [26]. A recent study [27] demonstrated that RanBP9 modulates the interaction of the AFT complex and can regulate whether this complex localises to nuclear spots [28] where transcription factories reside or nuclear speckles where RanBP9 may relocate AICD away from transcription factories [27]. AICD has been shown to compete with HDACs 1 and 3 for binding at the promoters of AB degrading enzymes neprilysin and transthyretin [29, 30].

Acetyltransferases such as Tip60 are promising therapeutic targets in comparison to deacetylases because specific acetyltransferases have less redundant targets within the genome and could be used to upregulate specific neuroprotective pathways [31]. For example, Tip60 overexpression can rescue AD drosophila from APP-induced learning and memory deficits [32, 33] and can also regulate the transcription of genes involved in a variety of neuronal processes [34]. A loss of Tip60 leads to axonopathy and aberrant histone acetylation-mediated expression of axonal transport genes [35]. Other acetyl transferases that have been shown to play a significant role in neuroprotection include p300 and CBP [36–38], and a critical role in mediating memory consolidation [39]. However, another study has suggested that CBP and p300 knockout mice are resistant to amyloid beta-mediated toxicity [40].

17.2.3 Global Histone Modifications Observed in PM Tissue to Date

Literature on global histone modifications in post-mortem AD brain is limited and varies significantly with regard to the methodology used, brain regions studied and sample sizes. Table 17.1 and Fig. 17.1 highlight the relevant studies published to date.

Increases in global histone modifications that would result in transcriptional activation in AD brain include trimethylation at histone H3 lysine 4 (H3K4Me3); acetylation at histones H3K9, H3K14, H3K18, and H3K23 and histone H4 lysines 5, 8, 12 and 16; as well as phosphorylation at histone H3 serine 10. In contrast, an increase in di-methylation on histone H3 lysine 9 is a signature of heterochromatin and results in transcriptional repression. Transcriptionally activating global histone changes in post-mortem AD brain have been observed in different regions of the brain, frontal cortex [41, 42], hippocampus [43–45], middle temporal gyrus [44, 46], inferior temporal gyrus [46] and occipital cortex [47], while transcriptionally repressive changes for AD brain have also been observed in temporal lobe [48], occipital lobe [47] and hippocampus [43]. It is difficult to reach a consensus from this data and begs the development of universal standards for tissue preparation and fixation, minimum sample size, developing robust techniques for addressing cellular heterogeneity, standardising imaging methods and the equipment/software used for quantification to enable more robust comparisons to be made between independent groups. Given the range of different methods and sample sizes used to study global histone changes,

Table 17.1 An overview of the studies to date that have investigated global histone modifications in PM-AD brain

First author	Title	Sample size		Methods	Regions	Overall change in AD relative to control
		Control	AD			
Rao et al. [41]	Epigenetic modifications in frontal cortex from Alzheimer's disease and bipolar disorder patients	10	10	Epigenetic global H3 acetylation and global H3 phosphorylation ELISA kits	Frontal cortex	No change in AcH3, increase in H3 phosphorylation (specific residues not specified)
Zhang et al. [48]	Targeted proteomics for quantification of histone acetylation in Alzheimer's disease	4	6	Selected reaction monitoring-based proteomics and Western blots to study H3K18/K23 acetylation	Temporal lobe	Significant decrease in H3K18/K23 acetylation
Chaput et al. [98]	Potential role of PCTAIRE-2, PCTAIRE-3 and P-histone H4 in amyloid precursor protein-dependent Alzheimer pathology	4	5	Western blots	Undefined	Significant increase in H4Ser47 phosphorylation
Mastroeni et al. [44]	Aberrant intracellular localisation of H3k4me3 demonstrates an early epigenetic phenomenon in Alzheimer's disease	19	18	Global IHC, WB and ELISA	Hp and MTG	H3K4Me3 colocalisation with NFTs significantly increased, nuclear labelling reduced

(continued)

Table 17.1 (continued)

First author	Title	Sample size		Methods	Regions	Overall change in AD relative to control
		Control	AD			
Narayan et al. [46]	Increased acetyl and total histone levels in post-mortem Alzheimer's disease brain	28 (MTG); 17 (ITG)	29 (MTG); 14(ITG)	Global IHC-free floating sections for ITG, IHC-P for tissue microarray of MTG and WB	ITG and MTG	Significant increase in AcH3 (K9/K14) and AcH4 (K5/K8/K12/K16) and proportional increase in respective total histone protein also
Hernández-Ortega et al. [43]	Altered machinery of protein synthesis in Alzheimer's: from the nucleolus to the ribosome	18	49	Global IHC-P	Hp	Observed decrease in H3K9Me2 and H4K12Ac
Lithner et al. [47]	Disruption of neocortical histone H3 homeostasis by soluble Aβ: implications for Alzheimer's disease	3	7	Histones isolated with nuclear extraction kit (Pierce Biotechnology) and analysed with WB	Occipital Ctx	Significant increase in AcH3K14 and H3K9Me2
Ogawa et al. [45]	Ectopic localisation of phosphorylated histone H3 in Alzheimer's disease: a mitotic catastrophe?	9	17	IHC-P and WB	Hp (IHC-P) and cortical grey matter for WB	Significant increase in H3Ser10 phosphorylation, staining appeared to colocalise with tangles
Lu et al. [42]	REST and stress resistance in ageing and Alzheimer's disease	11	8	IHC in isolated neuronal nuclei	PFC	Significant increase in AcH3K9 in AD compared to control cases

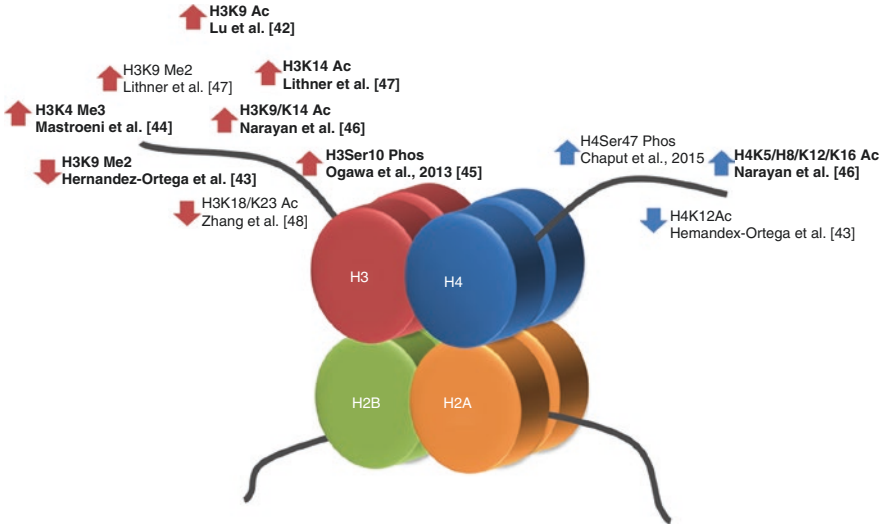

Fig. 17.1 A schematic overview of studies that have examined global histone modifications in PM-AD brain. The modifications that appear in *bold* delineate modifications that would result in transcriptional activation in AD cases

to be able to draw conclusions from similar studies, we focused below on reviewing studies that used immunohistochemical techniques and a sample size of ten or more cases in each control and AD group.

Mastroeni and colleagues [44] performed immunohistochemical analysis of hippocampal tissue from control (n = 19) and AD (n = 18) cases to analyse global changes in histone H3 lysine 4 trimethylation (H3K4Me3). H3K4Me3 is widely accepted as an epigenetic signature for actively transcribed or poised (waiting for transcriptional activation) genomic regions. They showed a reduction in nuclear labelling and an increase in cytoplasmic labelling—significantly colocalising with hyperphosphorylated tau tangles, in AD brains compared to non-demented controls. When cases were analysed by Braak stage, they showed that cytoplasmic localisation of H3K4Me3 preceded the earliest observations of tau hyperphosphorylation at epitopes known to be early markers of AD (PG5 and MG1 which detect phosphorylation at serines 409 and 312–322, respectively) [49], suggesting that intracellular localisation of this histone marker may be important in altering transcription in AD. They also demonstrated that the cytoplasmic staining increased in a manner corresponding to neuropathology (increasing Braak stage).

Narayan et al. [46] used immunolabelling of free-floating AD (n = 14) and control (n = 17) ITG sections and of tissue microarrays containing paraffin-embedded MTG from 28 control and 29 AD cases. Their results showed a significant increase in not only histone H3 and H4 acetylation but also corresponding increases in the total protein loads of histone H3 and H4. They found that each marker correlated significantly with levels of gliosis (HLA for microglia and GFAP for astrocytes) and with neuropathological hallmarks (tau and amyloid load) in AD but not control

cases. Significant correlations with ubiquitin load and each of the histone markers reinforced their hypothesis that protein degradation is compromised in AD and may cause the observed changes in histone markers, bringing into question the therapeutic efficacy of drugs that target the epigenome alone in AD.

Hernández-Ortega and colleagues [43] investigated whether major nucleolar proteins (which act as histone binding chaperones) were altered in AD in relation to histone markers H3K9Me2 and AcH4K12. Using immunohistochemical techniques on hippocampal tissue from 18 control and 49 AD cases (of mixed Braak stages), they found that decreases in the nucleolar proteins corresponded to decreases in H3K9Me2 and AcH4K12 levels in the hippocampus. They unfortunately did not show correlations of this relationship; however, they did show that the loss of nucleolar proteins increased with Braak stage.

17.2.4 ChIP Sequencing for Histone Markers in AD

Gjoneska and colleagues [50] studied a CK-p25 mouse model of AD compared to CK wild-type littermates to conduct chromatin immunoprecipitation sequencing (ChIP-Seq) experiments of seven different histone markers. They included markers associated with active promoters (H3K4Me3), those associated with enhancers (H3K4Me1 and H3K27Ac), or associated with repression (H3K27Me3 and H3K9Me3), markers associated with gene bodies (H3K36Me3 and H4K20Me1). Upregulated genes (3667) corresponding to H3K4Me3 peaks (relative to controls) were found to be enriched for immune and stimulus response functions, while downregulated genes (5056), corresponding to H3K4Me3 decreased-level peaks, were enriched for synaptic function and learning. Immune regulators that were identified to have an increased level of H3K4Me3 peaks included NFkB and PU.1 consistent with previous findings where PU.1 has been shown to regulate microglial activation and proliferation in AD [51].

This study however is the first of its kind in the context of AD, and this area begs more research particularly using neuronal specific ChIP-seq data generated from control and AD post-mortem human brain samples.

17.3 Therapeutic Implications

Over the past few years, the results of a number of animal studies have shown that in models of AD there is a consistent **reduction in histone acetylation**. This reduced histone acetylation is associated with cognitive changes, and both the histone ‘defect’ and the cognitive changes can be reversed using common inhibitors of histone deacetylases. We will review this literature and then compare these animal studies to results in human brain to determine whether they can be translated into effective therapies for AD.

Using an AD transgenic mouse expressing the Swedish double mutation of APP, Ricobaraza et al. [52] found a dramatic reduction in acetylation of histone H4 in

lysates of frontal cortex and in the hippocampus using immunohistochemistry. This reduced H4 acetylation was almost completely reversed by the HDAC inhibitor phenylbutyrate, which also reversed memory deficits in these mice. This reduced acetylation was only present in primary neuronal cultures grown from AD transgenic mice and reversed in vitro by phenylbutyrate. These authors also found that phenylbutyrate reduced tau phosphorylation in AD transgenic mice, but did not modify amyloid load. These results support the hypothesis of a hypoacetylation mechanism underlying AD, at least in a mouse model. Similar overall results were seen in a study by Francis et al. [53] using a different AD transgenic model in mice. Using an associative learning model, they found that wild-type mice showed increased acetylation of histone H4 24 h after learning, whereas AD mice showed reduced learning-associated H4 histone acetylation. Trichostatin A, a HDAC inhibitor, rescued both the histone acetylation defect and the memory performance of the AD mice. Using another HDAC inhibitor, sodium butyrate, Govindarajan et al. [54] also found that increasing histone acetylation in an AD mouse model alleviated both the hypoacetylation and memory function. Graff et al. [55] using the CK-p25 mouse model of neurodegeneration showed elevated HDAC2 levels correlated with reduced cognition and histone acetylation. They reversed both with a siRNA to HDAC2. Yao et al. [56] found that the HDAC inhibitor valproic acid reversed hypoacetylation of histones H3 and H4 and improved memory performance in AD transgenic mice. Qing et al. [57] also found VPA treatment rescued amyloid pathology and memory deficits in APP23 transgenic mice.

Using a high fat diet model of insulin resistance associated cognitive deficits, Sharma and Taliyan [58] found that the cognitive deficits in these mice were accompanied by histone H3 hypoacetylation (and reduced BDNF levels). The HDAC inhibitor SAHA reversed both the hypoacetylation (and elevated BDNF) and improved cognition. SAHA (vorinostat) was also used by Benito et al. [59] who showed that this drug improved cognition, reversed hypoacetylation of histones and had an anti-inflammatory effect on AD transgenic mice.

Cuadrado-Tejedor et al. [60] found that a combination of SAHA and phosphodiesterase 5 inhibitor tadalafil synergistically increased acetylation of histone H3 on the lysine 9 residue in APP transgenic mice. They speculated that the tadalafil might have augmented histone acetylation by driving the CBP histone acetyl transferase. This drug combination also enhanced LTP in hippocampal slices and improved memory in vivo. Pavlopoulos and colleagues [61] have also demonstrated a critical role for CBP-driven histone acetylation in maintaining memory in AD. They identified a histone binding protein RBAp48 to be significantly depleted in the entorhinal cortex and dentate gyrus of post-mortem AD cases. RbAp48 is an important regulator of CBP and plays a key role in modifying histone acetylation patterns in the brain. They developed a transgenic mouse model that expressed a mutant form of RbAp48 (lacking 54 N-terminal amino acids which are critical for its interaction with histone H4). The mutant mice displayed impaired memory and cognitive performance which correlated with loss of CBP-mediated histone acetylation, which was rescued when RbAp48 was reintroduced in the dentate gyrus via viral vector [61].

Thus, there is consistent and compelling data to suggest that in mouse transgenic models of AD, as well as in other mouse models of memory impairment, histones H3 and/or H4 are hypoacetylated. This leads to reduced expression of plasticity associated genes and may be responsible for memory impairment in these models. A range of non-specific HDAC inhibitors such as valproate, SAHA, sodium butyrate and phenylbutyrate reverse the hypoacetylation and improve memory processes.

However, there is some data showing hyperacetylation of histones in models of AD. Walker et al. [62] found that neurons grown from AD transgenic mice showed increased H3 and H4 histone acetylation compared to neurons from non-transgenic mice. They further showed that amyloid could increase acetylation in neurons in vitro from non-transgenic mice indicating that amyloid load may have been responsible for these effects. Guo et al. [63] used an indirect in vitro model of cellular stress-induced amyloid production in human neuroblastoma cells to show that increased amyloid production correlated with hyperacetylation of histones, perhaps mediated by decreased HDACs and increased HATs (CBP). Using another indirect in vitro model of hydrogen peroxide-induced amyloid production in human neuroblastoma cells, Gu et al. [64] found similar results. Finally, transfection of rodent neuroblastoma cells with a mutant APP also generated increases in gene-specific (PS1, BACE1) histone acetylation, possibly by enhancing the HAT p300 [65]. This in vitro data showing that elevating amyloid levels increases histone acetylation is supported by the work of Narayan et al. [46] who found that increased histone H3 and H4 expression and acetylation was correlated with amyloid load in AD brains.

Thus, there is evidence for both hyper- and hypo-acetylation in cellular and in vivo models of AD, although the overwhelming results of in vivo rodent AD models is clearly hypoacetylation. Furthermore, HDAC inhibitors in animal models of AD reverse the hypoacetylation defect and improve cognition.

Does this work using in vivo rodent models of AD translate to the clinic? A search of the clinical literature where HDAC inhibitors such as valproate have been used to treat AD is rather disappointing [66–74]. Of the most recent publications in this area, dose appears to be a significant factor; higher doses were not tolerated well, causing significant adverse side effects in AD patients, particularly in patients displaying symptoms of aggression and agitation [75–77].

Why then does it appear that this very convincing rodent work does not translate, at least based on current clinical data, to humans with AD? This question has plagued neuroscience research in general for many decades now, and this is not the place to discuss this in depth. The reader is referred to a number of publications discussing these issues [78–82]. However, there is data showing that one widely used HDAC inhibitor valproate shows species-specific pharmacological activity. Many years ago, we showed that valproate when added to rodent microglia induces caspase 3-mediated apoptosis [83] and also in surviving microglia stimulates their phagocytic activity against amyloid peptides [84]. However, when human microglia are exposed to valproate, there is no evidence of apoptosis, and phagocytosis of amyloid peptides is inhibited by valproate [85], at concentrations that greatly enhance acetylation of histone H3 and H4. These results suggest that perhaps HDAC

inhibitors have species-specific actions. Many more studies are required using different inhibitors to confirm this hypothesis, but this might explain the lack of effects of valproate on AD in the clinic.

As discussed above, Narayan et al. [46] found that increased histone H3 and H4 expression and acetylation in AD brains was correlated with amyloid load. Furthermore, histone increases also strongly correlated with ubiquitin load, suggesting that compromised protein degradation in AD brains might also contribute to increases in histones. Indeed, Narayan et al. [46] found that the proteasome inhibitor MG132 elevated ubiquitin levels and acetylation of histone H3 in human neuroblastoma cells. Interestingly, when valproate was combined with MG132, as expected, there was an increase in histone acetylation, but unexpectedly there was also an increase in ubiquitination and cell death. This result, if applicable to humans in the clinic, would suggest caution when using HDAC inhibitors to 'treat' AD.

17.4 Concluding Remarks

There has been some progress made in literature in moving from reductionist or global approaches of studying histone modifications, in the context of AD, towards methods that allow visualisation of epigenetic marker peaks scattered throughout the genome. However, there is significant scope for improvement. Moving forward, it will be essential to utilise international coordinated efforts such as the ENCODE project [86, 87], GENCODE [88], the National Institutes of health Roadmap Epigenomics Project [89] and the Broad Institute Reference Epigenomic Mapping Centre, in understanding and deciphering the epigenomes role in transcriptional regulation/dysregulation and AD. A major area of improvement in these databases will be not only brain region-specific mapping but shifting to purified cell populations and subpopulations. Using cell sorting or nuclei sorting methods prior to sample isolation will be essential. Also, sample sizes are small, and while efforts are being made to analyse multicentre cohorts of control and AD brain, there is room for improvement in this area.

In addition, utilising tools that will allow the analysis of crosstalk between histone and DNA modifications will be really important, and study designs should consider the use of sequential ChIP BS, ox-BS sequencing methods [90, 91]. Also incorporating methods that will address the contributions of non-CpG methylation [92–94], methylation of RNA (c5) [95], N1-methyladenosine [96] and hydroxymethyl RNA [97] in the context of AD will be of great interest.

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