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Blood DNA methylation as a potential biomarker of dementia: A systematic review

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Abstract

Dementia is a major public health issue with rising prevalence rates, but many individuals remain undiagnosed. Accurate and timely diagnosis is key for the optimal targeting of interventions. A noninvasive, easily measurable peripheral biomarker would have greatest utility in population-wide diagnostic screening. Epigenetics, including DNA methylation, is implicated in dementia; however, it is unclear whether epigenetic changes can be detected in peripheral tissue. This study aimed to systematically review the evidence for an association between dementia and peripheral DNA methylation. Forty-eight studies that measured DNA methylation in peripheral blood were identified, and 67% reported significant associations with dementia. However, most studies were underpowered and limited by their case-control design. We emphasize the need for future longitudinal studies on large well-characterized populations, measuring epigenetic patterns in asymptomatic individuals. A biomarker detectable in the preclinical stages of the disease would have the greatest utility in future intervention and treatment trials.

Keywords

Alzheimer's disease; Biomarker; Blood; Dementia; DNA methylation; Epigenetics; Peripheral

1. Introduction

Dementia is a substantial public health issue with huge social and economic burden. Approximately 47 million people worldwide were living with dementia in 2015, and this is estimated to rise to 131 million within the next 35 years [1]. The most common cause of

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dementia is Alzheimer's disease (AD), a progressive and highly debilitating neurodegenerative disorder accounting for two-thirds of dementia cases [2]. AD is characterized by the buildup of extracellular amyloid β ($A\beta$) senile plaques and intracellular neurofibrillary tangles—aggregates of hyperphosphorylated tau protein. A definitive diagnosis of AD can only be made using neuro-pathological techniques postmortem [3].

Accurate and timely diagnosis of probable AD in living individuals is challenging. The most commonly used criteria recommend detailed clinical examinations and neuropsychological testing, a comprehensive medical history, laboratory tests on cerebrospinal fluid (CSF) and/or blood [4], and imaging (i.e., magnetic resonance imaging [MRI], positron emission tomography [PET], and/or computerized tomography [CT]) [5,6]. However, these are time consuming and expensive, with a heavy burden on the individual, and require a specialist's knowledge for their interpretation. Indeed, many cases of dementia remain undiagnosed—almost half of all individuals in high-income countries and up to 90% of those living in low- and middle-income areas [1]. Furthermore, it is now clear that there is a long preclinical phase of AD and identifying individuals in this asymptomatic phase would not only permit earlier interventions but may also be crucial to the success of existing and future therapeutic treatments [7].

A major area of current research has thus been the search for robust and readily accessible biomarkers of AD. To date, most attention has focused on brain imaging and CSF biomarkers that target key neuropathological features of the disease, such as brain $A\beta$ and neurodegeneration. Promising biomarkers for AD include heightened brain $A\beta$ detected with PET and cerebral atrophy observed with MRI. A decline in $A\beta$ and increase in CSF tau have also been observed [8]. Importantly, these biomarkers have been observed in individuals before the emergence of clinical symptoms. However, despite their relative predictive accuracy, none are yet considered as diagnostic biomarkers for routine use in clinical practice [9]. One of the reasons is the lack of specificity of these biomarkers for distinguishing preclinical dementia from cognitive decline that is considered a normal part of aging [10,11]. Individuals positive for these biomarkers also display varying trajectories of disease progression that appear to be influenced by genetic and lifestyle factors [10]. Other major limitations with these biomarkers are their cost, invasiveness, and burden for individuals, thus limiting their usefulness for population-wide screening.

Further research has thus focused on identifying a robust biomarker of dementia, which can be measured in peripheral tissue and could be incorporated into routine screening, being noninvasive, widely accepted by individuals and relatively inexpensive to measure. Epigenetic biomarkers represent a potential candidate, and research in this area has expanded greatly over the last decade.

Epigenetics describes dynamic molecular modifications “above the level of the DNA,” which do not alter the genetic code but influence the way the genes encoded by the DNA are expressed. These molecular modifications, some of which are sensitive to underlying genetic variation, are often sensitive to environmental factors or are influenced by disease state [12]. Despite the tissue specificity of epigenetics, peripheral blood-based epigenetic biomarkers have shown promise in several fields of medical research and diagnostics. Some of the

strongest and most consistent results include the finding that smoking alters DNA methylation of specific genes and can be detected in peripheral blood [13]. DNA methylation signatures derived from peripheral blood have also been used to predict mortality [14,15]. Other more preliminary findings include hypomethylation of a particular gene in blood as a precancer biomarker [16], and depression may be associated with specific DNA methylation changes in peripheral tissue [17], despite it being a brain disorder. This growing trend has also given rise to studies that have investigated the potential for peripheral epigenetic biomarkers of AD. Given the exponential rise in research in this field over the last decade, it is timely to conduct a systematic review of the current state of this research.

The aim of our study was to determine the potential for a peripheral epigenetic biomarker of dementia, by conducting a systematic review of studies undertaken in this area to date.

2. Methods

The protocol for this systematic review was registered with the International Prospective Register of Ongoing Systematic Reviews (PROSPERO) [18], registration number: CRD42017067306 [19]. This systematic review was conducted following PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analyses) [20] with the relevant checklist included in Supplementary Table 3. For brevity, the detailed description of the full methods used, including the selection criteria, search strategy, manuscript selection, data extraction, and the critical assessment of each manuscript, are shown in Supplementary Materials 1 and Supplementary Tables 1 and 2. In short, we included any human studies published in English up until April 2017, which measured peripheral epigenetic biomarkers and compared these between individuals with and without dementia.

3. Results

3.1. Search results

A total of 375 studies were identified through the original search after removing duplicates (Fig. 1) [20], and a further 184 studies were excluded as they were found not to be relevant. This left 191 eligible articles for full-text assessment. At this stage, we further excluded reviews, editorials, conference abstracts, and animal and *in vitro* studies. We also excluded studies that did not assess dementia or measure DNA methylation. All remaining articles (n = 48) fulfilled the following inclusion criteria: (1) DNA methylation was measured in peripheral blood and (2) compared between individuals with dementia and another group [21–68]. Included studies were published between 2008 and 2017. A summary of the extracted data is shown in Table 1.

3.2. Characteristics of included studies

3.2.1. Study design—Of the 48 studies, 44 were case-control studies (ranging between 2 and 356 participants), which included two studies that used biobanked samples in a case-control design [29,41]. One case-control study of 1177 participants involved only a subsample of participants in their analysis, but the exact number was unclear [61]. Two of the studies were cohorts (with 52 and 71 participants, respectively) [33,57], and there were two small case reports (n = 6 and 3, respectively) [66,68].

3.2.2. Participants—Participants in most of the studies (92.6%) were in late-adulthood or older age, with the average age ranging between 57.8 and 83.8 years. Four studies also included controls from a younger healthy population (average age ranged between 21.1 and 21.7 years) [50,51,55,56]. One case report study included a 27-year-old individual, and two siblings of undisclosed age [68]. The age of cases and controls was not clearly stated in 10 other studies [29,33,47,59,61,62,64, 66–68].

Most studies included males and females (ranging from 9% to 78% females), however, there were two studies of females only [22,45] and one study of males only [34]. Nine studies did not state the sex of study participants [29,38,44,55,61,62,66–68].

Populations were diverse in ethnicity and described as Italians [21,27,35,37,48,49,60,65], Japanese [24,31,32,42,58], European [33,42], Chinese [45,68], Han Chinese [23,38], and Caucasian [66]. Another four studies included individuals of mixed ethnicity [42,59,65,67]. However, in 27 studies (56%), ethnicity was not mentioned.

One study [61] provided no information about the numbers of participants or their characteristics.

3.3. Dementia assessment

Most studies (75%) focused on AD, nine of thirty-six studies that specifically mentioned late-onset AD, although in all studies individuals with AD were aged over 65 years. These studies all compared individuals with AD to older individuals without a diagnosis, and as previously mentioned, four studies also included a younger healthy control group. One study investigated all-cause dementia in older individuals [57] who were followed over time, and another study included a group of participants with vascular dementia (VD) [25]. Five case-control studies [26,59,61,62,64] and one case report [68] involved individuals with frontotemporal dementia (FTD), three of which also included a group with amyotrophic lateral sclerosis (ALS) [61,62,64]. Two separate studies investigated dementia with Lewy bodies (DLB) [54,58].

Most studies (54.2% of all included studies and 72.2% of AD-related studies) used National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria for the diagnosis of probable dementia or AD. This was commonly in association with the Diagnostic and Statistical Manual of Mental Disorders (III or IV revision) (22.9% of studies). The most frequently used tools to support this diagnosis included the Mini–Mental State Examination (used in 56.3% of studies), although in the vast majority of cases detailed diagnostic information was not provided. Eleven studies reported the use of CT, MRI, or PET in diagnosing dementia-related disease [22,25,26,30,45,48,52,53,57,68]. Three studies made no specific mention of the diagnosis criteria used [29,40,59].

A number of studies investigated individuals with rare genetic diseases (where dementia was a principal symptom) and compared these individuals to family members without the gene mutation. There was a case study that observed an individual with a *MAPT* mutation [68]. Mutations in *MAPT*, which encodes microtubule-associated protein tau and is an integral

part of the development of the nervous system, are a major cause of FTD. Two studies were focused on hereditary sensory and autonomic neuropathy type 1 with dementia and hearing loss (HSAN1) [66,67], whereas five studies investigated a gene mutation in the chromosome 9 open reading frame 72, of which G₄C₂ repeats are thought to be the most common genetic cause of ALS and FTLTD [61–65]. In all cases, these diagnoses were based on medical records.

3.4. DNA methylation measure

The vast majority of studies were cross-sectional, and blood was collected at the same time when dementia was assessed. One prospective study [57] measured DNA methylation at follow-up and determined the association with a prior dementia diagnosis. Only one study collected blood samples at baseline, before dementia diagnosis [33].

All studies extracted DNA from peripheral blood, the majority using whole blood (28 studies, 58.3%), of which only two were not cross-sectional. Some studies reported the use of white blood cells, described specifically as leukocytes in eight studies [25,34,45,46,50,51,58,68]; mononuclear cells in six studies [22,26,35,36,44,52]; lymphocytes in two studies [43,47]; and CD4⁺ T cells [33], B cells [66], and monocytes [40] in one study each.

The methods for measuring DNA methylation varied widely with 24 different methods described. The most common was pyrosequencing, which was used in 13 studies [23,24,26,28,31,32,35,38,47,53,58,67,68], which is a direct-sequencing technique enabling single-nucleotide resolution for short DNA stretches. The other common technique for targeted gene analysis is matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MassARRAY; Sequenom EpiTYPER) and was used in three studies [42,50,51].

Only one study used whole-genome sequencing with Hi-Seq 2000 [66]. Nine studies used chip array technology that enabled simultaneous measures of thousands of loci in a hypothesis-free approach, to investigate methylation patterns throughout the genome. Six studies used the Illumina Infinium HumanMethylation450 BeadChip (HM450K) [28,29,33,41,57,59], and two studies used the smaller Infinium HumanMethylation27 BeadChip (HM27K) [30,67], which measure methylation at >450,000 and >27,000 5'-Cytosine-phosphate-Guanine-3' (CpG) sites, respectively. Finally, one study used a much smaller array containing 22 genes, the DNA Repair EpiTect Methyl II PCR Array [21].

3.5. Critical appraisal

We performed a critical appraisal of all the studies using established and widely employed criteria—the Joanna Briggs Institute Critical Appraisal tools [79]. This enabled an unbiased systematic evaluation of the quality of evidence from each study. Full details are provided in Supplementary Table 2.

The two cohort studies [33,57] and the two case reports [66,68] all scored quite highly (71.4%, 92.9% and 80%, 90%, respectively), when critically evaluated against the defined criteria [79]. The majority of studies were case-control, scored from 16.7% [64] to 94.4%

[25,37] in their critical appraisal, with the mean being 54.2%. Most used validated tools to measure DNA methylation (93%, Q8 on the criteria checklist) and assess dementia (86%, Q4), and 88.4% of studies used appropriate statistical analysis as far as could be assessed (Q10). However, many studies ($n = 32$) did not use the same criteria for the identification of dementia cases and controls (Q3), or detailed information on this was not provided (thus classified as “unclear” in this evaluation). Furthermore, confounding factors were not considered in 55.8% of studies (Q6), 16.3% did not provide sufficient detail to ascertain whether they had investigated confounding (Q7), and 69.8% did not adjust for any factors.

3.6. Summary of outcomes

Although 32 studies reported differences in methylation between individuals with dementia and those without, the high heterogeneity across studies, particularly in regard to the method of methylation measurement and genes investigated, prohibited any meta-analysis of the results. Instead, we report below a summary of the main findings.

3.6.1. Methylation differences between AD cases and controls—Overall, 36 studies compared methylation between individuals with AD and those without, with all but one [33] case-control study. In addition, a cohort study investigated all-cause dementia in older adults [57], with AD a likely cause in the majority of cases. Overall, 66.6% of AD studies reported methylation differences between the groups, but the genes investigated and those found to be associated varied widely.

3.6.2. Epigenome-wide association studies of AD—Of the seven studies using the HM450K for epigenome-wide methylation analysis, four focused on AD and one all-cause dementia (Table 2). One study ($n = 15$) found some small differences between groups (<5%) [33], and another ($n = 57$) reported 805 differentially methylated sites [29]. Both studies, however, failed to provide detailed information on the exact loci identified and/or the effects sizes and statistical significance. Loci in three genes (*DAPK1*, *GAS1*, and *NDUFS5*) were also reported to be differentially methylated in AD cases compared with controls ($n = 57$); however, no effect size or statistics were given [41]. Another study ($n = 12$) found 1021 differentially methylated loci, which differed in methylation between AD cases, individuals with mild cognitive impairment (MCI), and controls, and 156 of these were also associated with cognitive symptoms [28]. More specifically, this study found 4193 differentially methylated sites between controls and AD, but no further details were provided. The one cohort study ($n = 52$), investigating all-cause dementia also used the HM450K array and found an association between dementia and DNA methylation age ($\beta = 0.16$, $P = .019$), which is assessed using a combination of sites on the array [57].

Only one of the two studies that used the smaller HM27K array (methylation analysis at 27,000 sites, rather than 450,000) investigated AD ($n = 128$). They found lower average *HMOX1* methylation in association with AD compared with both MCI and control groups (77.94% $\pm$ 13.0 vs. MCI: 89.15% $\pm$ 6.5.9 and controls: 87.20% $\pm$ 3.1, no P value stated).

3.7. Global methylation studies

Four studies, including two on AD, only examined global methylation. AD was found to be associated with increased global methylation ($n = 81$, 67.10 ± 0.7 vs. 64.73 ± 0.6 , $P = .0122$), measured using luminescent methylation analysis [35]. The other study ($n = 194$) used an Imprint methylated DNA quantification kit MDQ1 and found no difference in methylation between AD cases and controls, but individuals with VD had increased methylation [25].

3.8. Common gene regions within AD studies

Genes that were commonly investigated in more than one study are listed in Table 3. The most consistently significant finding was an increase in *BDNF* methylation in individuals with AD compared to controls, in two studies of Asian populations ($n = 40$, $5.08 \pm 5.52\%$ vs. $2.09 \pm 0.81\%$, $P = .041$ [36]; $n = 106$, $9.50 \pm 4.43\%$ vs. $7.45 \pm 2.77\%$, $P = .004$ [38]). This, however, was not shown in a study of 40 Caucasians [34]. Two studies of *PIN1*, and using the same method to assess methylation, found a decrease in methylation in association with AD ($n = 60$, $3.76 \pm 0.89\%$ vs. $11.45 \pm 2.26\%$, $P = .001$ [48]; $n = 2$, 0.9% vs. 2.9% [22]). However, a third much larger study ($n = 317$) failed to find any differences [26].

Another consistency was seen in the reporting of null findings for a number of genes. All three studies of apolipoprotein E (*APOE*) ($n = 2$, 135, and 15, respectively) [22,46,47] and *MTHFR* ($n = 14$, 200, and 135, respectively) [21,27,37] and two studies each of *DNMT1* [27,37] and *DNMT3B* [27,37] ($n = 200$ and 135 respectively) reported no significant association between AD and methylation. A couple of other genes, *PSEN1* and *SIRT1*, were investigated in more than two studies. While the majority of studies reported no significant association, one study reported decreased *PSEN1* methylation and another *SIRT1* hypermethylation in association with AD, although no effect size or P value was given [27,46].

3.9. Other dementia-associated disease methylation studies

Further summaries on studies including FTD/FTLD, DLB, and genetic mutations leading to dementia may be found in Supplementary Materials 2.

4. Discussion

Overall 67% of all studies (32 of 48) reported a statistically significant difference in peripheral blood DNA methylation between individuals with dementia and those without, which included 67% of studies that examined AD (24 of 36). However, of these AD studies, very few investigated DNA methylation in the same gene, and none of the loci identified from epigenome-wide association were replicated across studies. The vast majority of findings came from small cross-sectional case-control studies in varying populations, and this may help account for the divergent findings. It remains uncertain whether peripheral DNA methylation marks differ between individuals with other causes of dementia, including FTD, DLB, and genetic mutations that result in dementia symptoms, due to the small number of studies undertaken to date.

4.1. Strength of evidence

This review was based on 48 studies including a total of 5689 participants. The average sample size of studies was relatively small, limiting analytical power. The 36 studies of AD had a total number of participants ranging between 2 and 283 (combined cases and controls), with a mean of 91. The median number of participants in studies of FTD was 102, and the five studies of *C9orf72* ranged in total number between 38 and 1177 individuals (including affected individuals, family members, and unrelated controls). The relatively small size of most of these studies precluded testing for sex-specific effects in stratified analyses, despite clear evidence of this in the broader field of epigenetics [80,81].

Overall, a small number of cohort studies and case reports were found to be quite robust and presented good-quality evidence. The majority of studies included in this review, however, were case control, and they were found to be variable in their quality. A key feature of case-control studies is that the individuals should be as similar as possible and recruited from the same overall population, differing only in their diagnosis of dementia. However, a large number of studies (46.5%) did not fulfill these criteria, and for others, it was unclear (27.9%). This is likely to result in selection bias, which may influence the results. Another area where several studies performed poorly was a failure to adequately consider potential confounding factors, as they were not sufficiently investigated or controlled for in >69% of studies. Given that DNA methylation is sensitive to many environmental influences and that individuals with dementia are likely to differ on a number of key characteristics (including medication history) compared to individuals without dementia, confounding by other factors may account (at least in part) for some of the findings.

4.2. Completeness and applicability of the evidence

Although 48 individually published studies were included in this review, a number of these came from the same research group, and often using very similar populations. This limits the overall generalizability of the results. For example, a total of seven AD case-control studies involved individuals recruited in Milan, Italy [26,35,44,48,49,53,60], and a further three studies also involved an Italian population [21,27,37]. Together these studies represent over 30% of all included studies of peripheral methylation and AD. There were also five studies involving Japanese individuals, three of which had very similar population characteristics and recruitment [24,31,32], and six studies of Chinese, two involving the same case-control cohort investigating different genes [50,51] and others with similar populations ([23,38] and [45,52]). Together this suggests that a large proportion of the findings in this review are based on the same or very similar groups of individuals.

Furthermore, ethnicity was not reported in over half (56%) of the studies presented. This, together with the failure to consider underlying genetic variation in all DNA methylation studies of AD included here, is also an important limitation given the known role of genetic variation in determining DNA methylation patterns. It is widely accepted that differential methylation can be associated with genetic variations, such as single-nucleotide polymorphisms [82] or variable number tandem repeats [83]. In this review, differential methylation was associated with the genetic repeats of *C9orf72* expansions [61,64].

However, there is a lack of studies that have combined genetic information and methylation marks when comparing individuals with dementia and those without.

4.3. Strengths and limitations of this review

This review offers a comprehensive summary of studies investigating peripheral blood DNA methylation in dementia. A strength of the search strategy was the inclusion of all possible causes of dementia, including rare genetic conditions that result in symptoms of dementia. We comprehensively extracted all relevant information from 48 identified studies and completed a quality assessment using recognized evaluation tools. Some limitations of the review are the inclusion of only studies published in English and which were identified from the two most comprehensive databases of published medical literature. Some published studies, however, may not have been included. We also need to consider positive publication bias, and those studies with negative results may not have been published and thus could not be included here. Finally, we decided to focus on DNA methylation, given the large scope of studies already published in this field, but cannot draw any conclusions regarding other possible epigenetic biomarkers of dementia.

4.4. Broader limitations of research in this field

Beyond the limitations of this review, *per se*, are a number of other important considerations to current research in this area more generally.

4.4.1. Complexity of dementia diagnosis—Dementia is an umbrella term used to describe a group of symptoms that result in long-term decline in cognitive functioning that is significant enough to affect daily function [84]. Diagnosis of dementia is complicated, and the underlying cause (e.g., AD, VD, or DLB) is not always clear. Yet these diseases are likely to have different etiologies and the associated epigenetic patterns may therefore also be quite different. Many studies in this review focused on AD; however, a definitive diagnosis can only be made postmortem. Studies have thus classified with possible/probable AD, often using NINCDS-ADRDA criteria, but without providing specific details on the neuropsychological tests, clinical exams, imaging, or blood tests, which were used. Misclassification of the underlying cause of dementia would have influenced some of the findings. However, this is a challenge that cannot be easily overcome and also one of the reasons driving the need for more research in this area.

4.4.2. Study design—To date, almost all studies have been case-control studies, where a group of individuals with dementia is recruited (together with a control sample) and DNA methylation measured at that same point in time. Such cross-sectional analysis is limited because of the lack of temporal information. DNA methylation marks could reflect or result from biological processes occurring only with overt clinical disease or at a threshold of disease severity. This has obvious limitations for its usefulness as a diagnostic biomarker. If an epigenetic biomarker is to have greatest utility, it must be detectable in the preclinical phase and thus identified in presymptomatic individuals. Longitudinal studies that collect biological samples at baseline and follow large groups of initially healthy individuals over time to track disease incidence are thus essential.

4.4.3. Tissue specificity of epigenetic marks—Dementia is a disease of the brain, and a number of studies have now shown epigenetic disruption in specific brain regions in individuals diagnosed with dementia [85]. An epigenome-wide association study (HM450K) of 708 dorsolateral prefrontal cortex brain samples, for example, identified 71 sites that were differentially methylated in association with AD pathology [86]. Eleven of these sites were validated in an independent sample (n = 117). However, there is little evidence to suggest that brain epigenetic modifications observed in AD will correlate strongly with epigenetic alterations observed in peripheral tissue. Indeed of the 12 studies included in this review, which measured DNA methylation in specific brain regions and in peripheral blood [29,33,40–42,47,50,51,61–64], only four studies reported methylation changes in both tissues, and this was likely to be driven by the underlying genetic repeats within *C9orf72* [61–64]. The majority of studies found no correlation between brain and blood methylation in dementia. For example, one study showed that *SORL1* and *SIRT1* methylation was not found to be significantly different between AD and controls in leukocytes or brain regions (entorhinal and auditory cortices and hippocampus) [50]; however, there was tissue-specific increased methylation between leukocytes and brain tissue in both groups (61 AD cases: +18.0%; 51 controls: +13.9%). Another more recent HM450K study (n = 41) also examined cross-tissue methylation differences and reported no strong evidence of a link between peripheral blood CD4+ lymphocyte methylation profiles and AD-associated methylation differences in the dorsolateral prefrontal cortex [33]. The methylation-specific profile of different cells also contributes to the complexity of analyzing brain tissue. Hypermethylation of *ANKK1*, for example, has been reported in the superior temporal gyrus and prefrontal cortex, but not in cerebellum (nor in peripheral blood) [41].

Together these findings suggest that dementia-associated DNA methylation changes detected in peripheral blood are unlikely to correlate directly with the neurodegenerative process of dementia. Yet there is evidence to indicate that these blood DNA methylation marks may mediate detectable transcriptomic changes [41], emphasizing their potential usefulness in clinical biomarker development. This is particularly the case given the difficulty in accessing brain tissue and inability to collect longitudinal brain samples to track disease diagnosis [87].

In addition to differences across tissues, the cell-specific nature of epigenetic patterns also needs to be kept in mind when interpreting the results of studies in this area. Although all studies in this review have measured DNA methylation in blood, only one study controlled for the estimated proportion of the different cell types, and none considered measuring methylation in individual cell types following sorting of the cells. Thus, it remains possible that differences in cell composition between groups, for example, an increase in monocytes in AD individuals compared with control groups [88], could drive some of the reported associations or mask potential differences between groups. Other peripheral tissues should also be considered in future studies. For example, buccal swabs are cheap and easy to collect and are thought to be closer in origin with brain tissue, having derived from the same primary germ cells within development [89]. No studies to date in this area, however, have examined DNA methylation of buccal tissue in AD.

4.4.4. Dynamic nature of DNA methylation—It has been well documented that DNA methylation is a dynamic epigenetic mechanism, especially in early development [90], and in certain disease states, somatic cells can also undergo de novo methylation changes [91]. These dynamic methylomic changes remain stable in the long term and have been used to classify specific subtypes of disease [92]. However, it remains unclear exactly how dynamic DNA methylation changes can be over shorter periods. Most research to date has provided only a static snapshot of methylation states in mixed cell populations [93], but there is some evidence that certain environmental exposures can elicit changes in blood methylation patterns over weeks [94] or even days [95]. Given that dementia has a long asymptomatic phase and generally slow progression rate, it is unlikely that short-term methylation differences would be observed. It is worth noting, however, that the time between collection of samples at baseline and follow-up in longitudinal studies and the time between sample collection and dementia onset or severity of disease state are potentially important considerations for future studies in this area.

4.4.5. Methods used to measure epigenetics—All studies included in this review measured DNA methylation, which is the most widely investigated epigenetic mechanism, with the most advanced analysis tools, thus enabling high throughput processing. This, combined with its relative cost effectiveness, makes it particularly appealing for cohort studies. However, the range of methods used to measure DNA methylation is vast [96,97], as are the individual laboratory processes, conditions, and methods of data analysis; including site-by-site analysis, regional methylation changes, preclustering or grouping of sites, and the identification of differentially methylated regions [98]. This large heterogeneity can make it difficult to directly compare findings, particularly with studies published more than a decade ago and because the field is expanding at such a rapid rate. There are ongoing attempts to help standardize these analysis approaches, which will be an important step in improving the reliability of findings and replication across studies [99]. The importance of internal and external validation of findings within a given study is also increasingly recognized. It is hoped this will lead to more robust research and minimize the number of chance findings (type 1 errors), which can never be replicated.

As mentioned, DNA methylation has been the predominant focus of studies not only in this area, but in the broader field to date. It is highly likely however that other epigenetic processes such as histone modifications and noncoding RNAs are also implicated in dementia and could also have potential as biomarkers [100,101]. For example, transcriptional dysregulation has been observed in Huntington's disease where histone acetylation is involved [102]. Clinical trials have also observed that histone acetyltransferase and histone deacetylase inhibitors have beneficial outcomes for those suffering from Huntington's disease. Further studies could also consider 5-hydroxymethylcytosine, which has been shown to modify tau-induced neurotoxicity [103]. However, their potential to also be blood biomarkers of dementia has not been established.

4.4.6. Comparison with other peripheral biomarkers—Current biomarkers that are used to aid in dementia diagnosis include procedures that are invasive in nature, such as PET and CSF collection. For a PET scan, the procedure is a lengthy and uncomfortable process.

Patients need to fast for at least 4 hours before an injection of a radioactive compound into their blood stream and then need to wait half an hour before imaging [104]. A lumbar puncture is required for the collection of CSF and involves penetrating a patient in the lower back with a large needle, which is extremely invasive and has adverse side effects [105,106]. Both these techniques are costly, require a specialist, and are performed in select locations. In contrast, a blood sample is relatively quick and easy to collect, with minimal side effects and much higher patient acceptability. Blood collection is already a routine part of regular medical checkups, and as such, this would greatly facilitate the implementation of general population screening if a promising biomarker was identified.

Beyond epigenetics, a number of other blood-based biomarkers are also being investigated but are beyond the scope of this review. This includes recent findings concerning apolipoprotein J and chemokine-309, which were both found to be increased in AD individuals compared with controls and those with MCI [107].

4.5. Considerations for future studies in epigenetic biomarkers of dementia

To advance research in this area, we need robust studies with strong methodological designs. Ideally, such studies would involve prospective recruitment of a large cohort of healthy individuals reflective of the wider population, with longitudinal follow-up and sampling over several decades, including regular and in-depth collection of demographic, lifestyle, and health data (enabling later adjustment for a wide range of potential moderating and confounding factors). Clinical assessments should include a comprehensive battery of neuropsychological assessments to track cognitive decline and the early signs of dementia. Dementia should be diagnosed and adjudicated by an expert panel using the results of cognitive tests, detailed medical histories, blood tests, clinical examinations, and with structural imaging. Blood samples should be collected at recruitment (prediagnosis) and across multiple time points, not only allowing for preclinical biomarker discovery but also monitoring of changes in these biomarkers with the occurrence of disease symptoms.

In terms of epigenetics, whole epigenome-bisulfite sequencing should ideally be performed to measure DNA methylation patterns at single base-pair resolution throughout the genome. This unbiased approach of interrogating sites throughout the entire genome would undoubtedly help uncover novel sites and genes of interest. This information could then be analyzed alongside genomic data, enabling important teasing out of epigenetic-specific associations from underlying genetic risk. Unfortunately, however, these kinds of comprehensive studies may be years off, due to the technical complexity of genome and epigenome sequencing, the complex analysis and importantly the prohibitive high costs, especially for large studies which are needed here.

Currently, a more viable option would be to use the newest technology in array-based analysis, the Infinium MethylationEPIC BeadChip, which provides methylation information for over 850,000 CpG sites throughout the genome [108]. Such a large amount of comprehensive data would allow for the creation of epigenetic risk profiles, akin to the genetic risk profiles, and the potential for a pre-clinical diagnostic marker. The main limitation of this platform, however, is that these arrays are still quite expensive, especially when obtaining data on a large number of participants at multiple time points. The field

however is progressing at an ever-increasing rate and with continual advances in epigenetic technologies, it is likely that new and improved approaches will also become available; however, the costs of these are again likely to be high [109].

5. Conclusion

This review has highlighted the gaps in our understanding of peripheral DNA methylation biomarkers of dementia, with as yet insufficient evidence to draw any clear conclusions. Currently, methods of methylation analysis and outcomes are highly heterogeneous, and the populations within these studies are mostly small or very small, deriving from a limited number of populations. This is a limiting factor in current research; however, the field is still in its infancy. The oldest article included in this review was published 9 years ago, with 90% of articles published in the last 6 years. Although the findings are variable and taking into account positive publication bias, it is worth noting that a number of studies did find differential methylation in association with dementia. It is likely that genomic and epigenomic analysis of genes would provide more comprehensive answers about the real utility of DNA methylation as a biomarker of dementia. Ideally, future analyses would have robust assessments of AD and related dementias and would employ longitudinal data with individuals both before disease onset and following clinical diagnosis. This work will further the research into clinically useful biomarkers and could potentially lead not only to concise and timely diagnosis of dementia but possibly to the discovery of predictive markers associated with preclinical disease stages. High sensitivity and specificity to differentiate AD from non-AD will need to be attained for this to have any potential as a clinically meaningful tool.

Dementia is an increasingly urgent public health issue, and research and technology continues to advance in the field of epigenetics. The findings of this review are important to summarize where the field is at and a key step that must be taken before we can advance this work further.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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RESEARCH IN CONTEXT

1. Systematic review: We completed a systematic review of electronic databases (e.g., MEDLINE, EMBASE) to identify human studies that had compared DNA methylation in peripheral blood between individuals with and without dementia.
2. Interpretation: Methylation differences were reported in 67% of the 48 studies identified, but very few of these studies meet high-critical appraisal criteria. The predominance of small case-control studies limits the interpretation of findings to date.
3. Future directions: There is a need for large prospective studies of initially healthy individuals with detailed clinical assessments to track cognitive decline and the very early signs of dementia, diagnosis of which should be adjudicated by an expert panel. Blood samples should be collected at recruitment (prediagnosis) and across multiple time points, and epigenome-wide DNA methylation measured. This hypothesis-free approach would not only allow for preclinical biomarker discovery but also monitoring of changes in these biomarkers with the occurrence of disease symptoms.

Fig. 1.
Flow diagram of manuscript selection.

Table 1
Studies investigating peripheral blood DNA methylation in association with dementia

Study	Recruitment and study design	Exclusion/Inclusion criteria	N, mean (SD) age, sex, ethnicity	Diagnosis	Tissue	Methylation platform	Gene region	DNA methylation finding	Adjustments
[21]	Case-control study, recruited from a University Hospital in Pisa, Italy	Controls had no cognitive impairment, familial history of any dementia	56 AD, 76.3 (7.3) years, 55% ♀ 55 controls, 77.9 (10.1) years, 58% ♀ Subset for array analysis: 7 AD, 79.1 (4.7) years, 71% ♀ 7 controls, 79.7 (5.2) years, 71% ♀ All Italian Caucasians	Probable AD diagnosed by DSM-IV and NINCDS-ADRD criteria. AD assumed sporadic LOAD based on age of onset and absence of family history	WPB	DNA Repair EpiTect Methyl II PCR Array and MSHRM promoter methylation	Array: <i>APEX1</i> , <i>LIG3</i> , <i>PARP1</i> , <i>POLB</i> , <i>UNG</i> , <i>XRCC1</i> , <i>CCNH</i> , <i>RAD23A</i> , <i>RAD23B</i> , <i>MRE11A</i> , <i>XPC</i> , <i>MLH1</i> , <i>MLH3</i> , <i>MSH2</i> , <i>PMS2</i> , <i>POLD3</i> , <i>ATM</i> , <i>BRCA1</i> , <i>BRCA2</i> , <i>FEN1</i> , <i>RAD50</i> , <i>RAD51</i> MS-HRM: <i>OGG1</i> , <i>PARP1</i> , <i>MRE11A</i> , <i>BRCA1</i> , <i>MLH1</i> , <i>MGMT</i> , <i>PSEN1</i> , <i>MTHFR</i>	No difference	Age, sex
[22]	Case-control of dementia discordant monozygotic twins recruited from an Italian geriatric unit.	None	1 AD, 70 years at evaluation, ♀ 1 control: 71 years at evaluation, ♀ Ethnicity not stated	AD diagnosed by NINCDS-ADRD criteria. Included MMSE, NDI, ADL, IADL, CT/ MRI, FDG-PET, and CSF	PBMC	MSP rPCR	<i>APOE</i> , <i>APP</i> , <i>PSEN1</i> , <i>SIRT1</i> /promoters	AD lower <i>PIN1</i> (0.9 vs. 2.9%) and higher <i>APP</i> (4.1 vs. 3.6%)	None mentioned
[23]	Case-control study, recruited from hospitals in Zhejiang, China	AD participants excluded if they had VD or depressive symptoms	51 sporadic AD, 80.94 (8.8) years, 47% ♀ 63 controls: 79.78 (7.9) years, 27% ♀ All Han Chinese	Sporadic AD assessed by ICD-10 criteria. Included MMSE, MoCA, and ADL	WPB	Pyrosequencing	<i>OPRD1</i> CpG island in promoter, 3 CpGs	AD increase at one site (77.96 ± 4.08 vs. 74.41 ± 3.98, <i>P</i> = 2.12E-04)	Sex, smoking
[24]	Case-control study, recruited from hospitals in Ehime, Japan	Controls without cognitive impairment or history of mental disorders	50 AD: 77.7 (6.1) years, 78% ♀ 50 controls: 76.3 (6.0) years, 78% ♀ All Japanese	Probable AD using NINCDS-ADRD criteria. Included MMSE, CDR, and NPI	WPB	Pyrosequencing	<i>ABCA7</i> promoter, 5 CpGs	No difference	mRNA expression level, age, sex, rs3764650 allele, <i>APOE</i> ε4/ε
[25]	Case-control study, recruited from neurology departments in Warsaw, Poland	Participants excluded with malignant disease, hypothyroidism, alcohol abuse, mood disorders,	53 AD: 70.9 (8.3) years, 79% ♀ 17 VD: 69.6 (6.9) years, 65% ♀ 32 mixed dementia: 74.5 (6.1) years, 72% ♀	AD diagnosed by NINCDS-ADRD criteria. VD by NINDS-AIREN criteria. Included MMSE, ICD-10, cognitive tests, and	PBL	Imprint methylated DNA quantification kit MDQ1	Global methylation	No difference. VD trend for increase compared with other groups (<i>P</i> = 5.061)	Age, sex, MMSE, various blood measures, serum apoE, and SNPs

Study	Recruitment and study design	Exclusion/Inclusion criteria	N, mean (SD) age, sex, ethnicity	Diagnosis	Tissue	Methylation platform	Gene region	DNA methylation finding	Adjustments
[26]	Case-control study, outpatients recruited from geriatric/neurological units in Milan, Italy	folate/vitamin B12 supplementation	47 MCI: 69.0 (8.2) years, 51% ♀ 45 controls: 71.8 (6.5) years, 56% ♀ Ethnicity not stated	CT/MRI, MCI assessment based on MMSE	PBMC	Pyrosequencing	<i>PIN1</i> promoter, 5 CpGs	FTD increase <i>PIN1</i> at 3 sites compared with AD and controls (−0.4%, $P < .03$). No differences between AD and controls	Age, sex
[27]	Case-control study, recruited from medical centers in Italy	Controls had no cognitive impairment, familial history of any dementia, not taking drugs supplements, vitamins	176 LOAD: 79.2 (0.5 SE) years, 73.3% ♀ 34 FTD: 73.5 (1.1 SE) years, 44.1% ♀ 107 controls: 79.7 (0.6 SE) years, 61.7% ♀ Ethnicity not stated	AD diagnosed by NINCDS-ADRDA criteria. Included MMSE, cognitive tests, and CT/MRI. FTD diagnosed by criteria [*]	WPB	MS-HRM	Promoter methylation: <i>PSEN1</i> , <i>BACE1</i> , <i>MTHFR</i> , <i>DNM1T1</i> , <i>DNM1T3A</i> , and <i>DNM1T3B</i>	Nonsignificant AD increase in <i>BACE1</i> (0.5 ± 0.9 vs. 0.8 ± 1.2, $P = .06$). Nonsignificant decrease in <i>PSEN1</i> (0.6 ± 1.2 vs. 0.9 ± 1.9, $P = .18$)	Map connectivity
[28]	Case-control study, outpatients recruited from hospitals in Japan	Controls had no cognitive impairment or history of mental disorders	30 AD: 71.8 (0.9) years, 53.3% ♀ 28 aMCI: 72.0 (0.9) years, 53.6% ♀ 30 controls: 70.5 (1.0) years, 60% ♀ Four from each for array. Ethnicity not stated	AD diagnosed by NINCDS-ADRDA criteria. aMCI diagnosed by criteria [†] . Included Behavior AD, MMSE, and FAB	WBC	HM450K and pyrosequencing for validation	485,577 CpGs, then 4 <i>NCAPH2</i> / <i>LMF2</i> CpGs for validation	156 differential loci associated with symptom progression. AD lower methylation at 4 <i>NCAPH2</i> / <i>LMF2</i> sites (between −2% and 5%, $P < .001$).	None mentioned
[29]	Stored samples at MRC London Neurodegenerative Disease Brain Bank, UK	None	48 AD, 9 controls, 83.2 years (range 70–96). Sex and ethnicity not stated	London Neurodegenerative Disease Brain Bank	WPB	WPB HM450K	485,577 CpGs	805 differentially methylated CpGs between groups	None mentioned
[30]	Case-control, recruited from health centers, Korea	Cases excluded with severe/unstable disease, very low B12/folate levels, positive syphilis serology, other neurodegenerative	105 AD: 81.26 (5.4) years, 75% ♀ 13 MCI: 73.62 (6.2) years, 46% ♀ 10 controls: 72.10 (7.4) years, 20% ♀	AD diagnosed by NINCDS-ADRDA criteria. Included MRI (brain atrophy, absence of vascular lesions), MMSE, CDR, and	WPB	HM27K, qMSP and Genome Sequencer FLX titanium (Roche/454 Life Sciences) for validation	27,578 CpGs <i>HMOX1</i> promoter 1 CpG	AD decrease in <i>HMOX1</i> (77.94 ± 13.0 vs. MCI 89.15 ± 5.9 and controls 87.20 ± 3.1)	Age, sex, <i>APOE</i> ε4/ε4

Study	Recruitment and study design	Exclusion/Inclusion criteria	N, mean (SD) age, sex, ethnicity	Diagnosis	Tissue	Methylation platform	Gene region	DNA methylation finding	Adjustments
[31]	Case-control study, recruited from hospitals in Ehime, Japan	disorder, and drug/alcohol addiction Controls had no cognitive impairment or history of mental disorders. Cases were not taking antidepressants	Ethnicity not stated 43 AD: 79.8 (3.8) years, 62.8% ♀ 47 controls: 79.7 (4.4) years, 66% ♀ All Japanese	neuropsychological battery Probable AD using NINCDS-ADRD criteria. Included MMSE, CDR, NPI	WPB	Pyrosequencing	<i>SLC6A4</i> promoter, 9 CpGs	No difference	None mentioned
[32]	Case-control study, recruited from hospitals in Ehime, Japan	Controls had no cognitive impairment or history of mental disorders. Cases excluded if they had cerebrovascular lesion	50 AD: 76.2 (5.8) years (5.8), 70% ♀ 50 controls: 75.9 (6.2) years, 70% ♀ All Japanese	Probable AD using NINCDS-ADRD criteria. Included MMSE, CDR, NPI, ADAS, and MADRS	WPB	Pyrosequencing	<i>SNCA</i> , 10 CpGs in intron 1	AD decrease on average (8.8 ± 2.7 vs. 9.5 ± 2.5 , $P = .027$) and at 7 sites.	None mentioned
[33]	ROS and RMAP, recruited participants primarily from retirement communities, USA. Mean FU 7.5 years	Excluded participants with known dementia.	41 participants: 81.2 (6.2) years, 65.7% ♀ 30 with AD at FU, 11 without. All European	Probable AD diagnosed by NINCDS-ADRD criteria. Pathological Alzheimer's based on neuritic plaque pathology	PB CD4+Tc	HM450K	14 candidate CpGs (brain methylation previously associated with AD pathology). Global methylation and gene-specific regions (420,132 CpGs)	No differences in candidate CpGs. Modest differences in global methylation (<5% median)	Age, sex
[34]	Case-control, recruited from community as part of longitudinal Conselice study in Ravenna, Italy	None	20 AD: 76 (7) years, 0% ♀ 20 controls: 74 (7) years, 0% ♀ Ethnicity not stated	AD diagnosed by NINCDS-ADRD criteria, according to DSM-III. Assessment included MMSE	PBL	MSP qPCR	<i>BDNF</i> , <i>SIRT1</i> , <i>PSEN1</i> /promoters	No difference	None mentioned
[35]	Case-control study, outpatients recruited from a geriatric unit in Milan, Italy	Cases excluded with sudden onset disorder/changes in behavior, other major disorders, or alcohol abuse. Controls had no cognitive impairments or disorders	37 LOAD: 80 (5.8) years, 59.4% ♀ 44 controls: 78.8 (6.7) years, 60.5% ♀ North Italian Caucasians	LOAD diagnosed by NINCDS-ADRD criteria. Assessment included MMSE	PBMC	Luminescent methylation analysis and pyrosequencing	Global methylation	LOAD increased global methylation (67.10 ± 0.7 vs. 64.73 ± 0.6 , $P = .0122$)	None mentioned
[36]	Case-control study, outpatients recruited from hospitals in Japan	Controls had no cognitive impairment or	20 AD: 66.5 (5.1) years, 60% ♀ 20 controls: 66.5 (5.1) years, 60% ♀	Probable AD diagnosed by NINCDS-ADRD criteria. Included	PBMC	Bisulphite PCR, ABI 3730DNAAnalyzer	<i>BDNF</i> -promoter of exon 1, 20 CpGs	AD increase (5.08 $\pm$ 5.52% vs. 2.09 $\pm$ 0.81%, $P = .041$) and at 1 site (4.85	None mentioned

Study	Recruitment and study design	Exclusion/Inclusion criteria	N, mean (SD) age, sex, ethnicity	Diagnosis	Tissue	Methylation platform	Gene region	DNA methylation finding	Adjustments
[37]	Case-control study, recruited from medical centers in Italy	history of mental disorders Controls had no cognitive impairment, familial history of any dementia, not taking drugs, supplements, or vitamins	Only 12 and 6 respectively for methylation analysis. Ethnicity not stated 120 AD: 76.9 (8.1 SEM) years, 59.2% ♀ 115 controls: 76.3 (8.3 SEM) years, 55.7% ♀ Italian Caucasians	Behave-AD, MMSE, FAB AD diagnosed by DSM-IV criteria, assumed LOAD. Assessment included MMSE	WPB	MS-HRM	<i>PSEN1</i> , <i>BACE1</i> , <i>MTHFR</i> , <i>DNMT1</i> , <i>DNMT3A</i> and <i>DNMT3B</i> promoters	AD nonsignificant increase in <i>BACE1</i> (0.62 ± 0.11 vs. 0.74 ± 0.11, <i>P</i> = .12), and nonsignificant decrease in <i>DNMT3A</i> methylation (0.90 ± 0.25 vs. 1.59 ± 0.26, <i>P</i> = .10)	Age, sex, homocysteine, folate, vitamin B12 and batch
[38]	Case-control study, recruited from hospitals in Zhejiang, China	Participants with familial AD excluded. Controls had no medical condition	44 AD: 80.0 (8.9) years 62 controls: 79.6 (7.9) years Sex not specified. All Han Chinese	Sporadic AD assessed by ICD-10 criteria. Included MMSE, MoCA, and ADL	WPB	Pyrosequencing	<i>BDNF</i> , 4 CpGs	AD higher average (9.50 ± 4.43 vs. 7.45 ± 2.77, <i>P</i> = .004) and at all sites	None mentioned
[39]	Case-control study in recruited from hospitals in Zhejiang, China	Controls with familial or personal history of neurological disorders excluded	28 LOAD: 77.4 (6.5), 67.8% ♀ 30 controls: 75.0 (6) years, 63% ♀ Ethnicity not stated	LOAD diagnosed by NINCDS-ADRDA criteria	WPB	MS-HRM	<i>LINE-1</i> general methylation levels	No difference	<i>APOE</i> ε4/ε sex
[40]	Case-control study, recruited from departments in Bonn, Germany	None	50 AD: 72.0 (7.1) years, 52% ♀ 55 controls: 67.7 (8.0) years, 49.1% ♀ Ethnicity not stated	Not mentioned	PBM	Bisulphite PCR, ABI PRISM 310 Genetic Analyzer	<i>TNF-α</i> promoter, 10 CpGs	No difference	None mentioned
[41]	Stored samples collected premortem at MRC London Neurodegenerative Disease Brain Bank, UK	None	48 AD: 83.8 years (7.0), 70.8% ♀ 9 Controls: 80 years (6.1), 66.7% ♀ Ethnicity not stated	AD clinical diagnosis. Details not given	WPB	HM450K	485,577 CpGs and more specifically <i>ANKK1</i> DMR	AD associated differentially methylated regions identified: <i>DAPK1</i> (cg14067233), <i>GAS1</i> (cg14067233), <i>NDUFS5</i> (cg17074958)	Age, sex
[42]	Case-control study. Brain samples from Douglas Hospital Research Centre	Controls without depression, stroke, VD, alcoholism, or low cognition. AD	34 AD: 75.47 (9.1) years, 73.5% ♀	Probable AD diagnosed by NINCDS-ADRDA	WPB	Sequenom EpiTYPER	<i>HSPA8</i> promoter, 22 CpGs and <i>HSPA9</i> promoter, 34 CpGs	AD mixed direction in <i>HSPA8</i> at 3 sites and AD decrease <i>HSPA9</i> at 3 sites	None mentioned

Study	Recruitment and study design	Exclusion/Inclusion criteria	N, mean (SD) age, sex, ethnicity	Diagnosis	Tissue	Methylation platform	Gene region	DNA methylation finding	Adjustments
	Brain Bank (Canada). No details about blood samples	with symptoms for 3 years	23 controls: 72.09 (10.5) years, 73.9% ♀ 95% European, 2.5% Japanese, 2.5% mixed	criteria and DSM-IV criteria					
[43]	Details not provided	Inclusion: 60 years men or postmenopausal, controls no family history of AD, genetic diseases, depression, VD, or other major diseases and MMSE 26	25 AD: 75.4 (9.3) years, 64% ♀ 25 controls: 75.4 (9.3) years, 64% ♀ Ethnicity not stated	Sporadic AD diagnosed by NINCDS-ADRDA and DSM-IV-R criteria	PBLy	Bisulfite PCR, sequenced with M13 primers	<i>DR4</i> promoter 2 CpG islands, 9 and 13 CpGs.	AD decrease in mean ($0.87 \pm 0.3\%$ vs. $2.18 \pm 0.4\%$, $P < .05$), island 1 ($2.04 \pm 0.71\%$ vs. $4.44 \pm 0.93\%$, $P < .05$) and 1 site (effect size not given, $P < .05$). Island 2 different at 2 sites (no effect size, $P < .05$)	None mentioned
[44]	Case-control study, recruitment details not provided	Controls had no neurological or psychiatric disorders, did not take psychotropic drugs and were independently living in the community	27 LOAD: 79.5 (6.30) years 28 controls: 80.0 (6.36) years Sex and ethnicity not stated	LOAD diagnosed by NINCDS-ADRDA criteria	PBMC	Fluorescence-based rtPCR, MS Opticon 2 Light Cycler instrument (Roche, Germany)	<i>Alox5</i> promoter	LOAD decreased (approximately 0.5-fold, $P = .0062$)	None mentioned
[45]	Case-control study, recruited from hospital outpatient clinics in Beijing, China	Participants excluded with any major complications or diseases, users of estrogen replacement therapy, vitamins treatment and smokers	23 AD: 71.6 (4) years, 100% ♀ 29 controls: 72.1 (4.9) years, 100% ♀ All Chinese	AD diagnosed by DSM-IV criteria, MMSE score 10-26 and CT	PBL	MspI and HpaII telomere length distributions	Subtelomeric DNA	No difference	None mentioned
[46]	Case-control study, recruited from hospitals in China	Participants with other major health conditions excluded. Controls had no neurological diseases	63 AD: 80 (11) years, ♀ 72 controls: 77 (15) years, 50.7% ♀ Ethnicity not stated	Probable AD diagnosed by NINCDS-ADRDA criteria	PBL	MSP, analyzed on 2% agarose gels and visualized under UV illumination	<i>SIRT1</i> , 3 regions covering CpG islands (SI, SH1, and SH2), promoter regions of <i>APP</i> , <i>APOE</i> e4/x, <i>PS1</i> , <i>PS2</i> , and <i>tau</i> .	AD hypermethylation of <i>SIRT1</i> (SI) (no effect size or P value given), increase of 7 of 11 sites and decrease in <i>APP</i> ($P < .05$, no effect size given)	None mentioned
[47]	Case-control study, recruited from Alzheimer's Disease	Controls included if 54 years, not diagnosed with AD	9 AD: 33.3% ♀, 6 controls: 66.7% ♀	AD diagnosed by CERAD criteria (moderate-to-frequent	WBL	Pyrosequencing	<i>APOE</i> exon 4, 75 CpGs	No difference	Tissue type, CpG site,

Study	Recruitment and study design	Exclusion/Inclusion criteria	N, mean (SD) age, sex, ethnicity	Diagnosis	Tissue	Methylation platform	Gene region	DNA methylation finding	Adjustments
[48]	Research Center, Washington, USA Case-control study, recruited from outpatients attending a geriatric unit in Italy	and no AD neuropathology at autopsy Participants with inflammation excluded. Controls had no neurological or cognitive disorder, other medical problems and were living independently in the community	Age at blood collection not given. Ethnicity not stated 32 LOAD: 79.5 (6.3) years, 71.9% ♀ 28 controls: 80.0 (6.4) years, 67.9% ♀ Caucasians from Northern Italy	neuritic plaques in neocortex and Braak stages V LOAD diagnosed by NINCDS-ADRD criteria. Assessment included CT/MRI, MMSE	WPB	MSP qPCR, MS Opticon 2 Light Cycler instrument (Roche, Germany)	<i>PIN1</i> promoter	LOAD decrease (3.76 ± 0.89 vs. 11.45 ± 2.26 , $P = .001$)	None mentioned
[49]	Case-control study, recruited from outpatients attending a geriatric unit in Italy	Participants with inflammation excluded. Controls had no neurological or cognitive disorder	33 LOAD: 79.47 (6.3) years, 70% ♀ 33 controls: 79.98 (6.4) years, 70% ♀ Caucasians from Northern Italy	LOAD diagnosed by NINCDS-ADRD criteria. Assessment included CT/MRI, MMSE	WPB	MSP qPCR	<i>FAAH</i> (mean from 18 CpGs)	LOAD decrease ($41.20 \pm 4.9\%$ vs. $55.90 \pm 4.6\%$, $P < .05$)	None mentioned
[50]	Case-control study, recruited from a university department in Sao Paulo, Brazil	Controls had no depression, stroke, VD, alcoholism, or poor cognition	36 AD: 75.4 (8.9) years, 73.9% ♀ 25 young controls: 21.7 (1.6) years, 76.0% ♀ 23 older controls: 72.1 (10.5) years, 69.4% ♀ Ethnicity not stated	Probable AD diagnosed by DSM-IV criteria and NINCDS-ADRD. Assessment included MMSE and Katz index	PBL	Sequenom EpiTYPER	<i>SORL1</i> (13 CpGs) and <i>SIRT1</i> (22 CpGs) promoters	No difference	None mentioned
[51]	Case-control study, recruited from a university department in Sao Paulo, Brazil	Controls had no depression, stroke, VD, alcoholism, or poor cognition	34 AD: 75.4 (8.9) years, 73.9% ♀ 22 young controls: 21.7 (1.6) years, 76% ♀ 23 older controls: 72.1 (10.5) years, 69.4% ♀	Probable AD diagnosed by DSM-IV criteria and NINCDS-ADRD. Assessment included MMSE and Katz index	PBL	Sequenom EpiTYPER	SNAP25 promoter	No difference	None mentioned
[52]	Case-control study, recruited from hospital outpatient clinics in Beijing, China	Participants excluded with any major complications or diseases, users of estrogen replacement therapy, vitamins	40 AD: 69.8 (5.1) years, 52.5% ♀ 59 controls: 70.1 (4.5) years, 49.2% ♀ Ethnicity not stated	AD diagnosed by DSM-IV criteria, MMSE score 10-26 and CT	PBMC	MspI and HpaII telomere length distributions	Subtelomeric DNA	AD had faster telomeric hypomethylation with aging (0.0435 vs. 0.0340 , no P value given)	None mentioned

Study	Recruitment and study design	Exclusion/Inclusion criteria	N, mean (SD) age, sex, ethnicity	Diagnosis	Tissue	Methylation platform	Gene region	DNA methylation finding	Adjustments
[53]	Case-control study. Cases recruited from Alzheimer unit in Milan, Italy. Control recruitment details not given	treatment and smokers None	43 AD: 75 (8) years, 53.5% ♀ 38 controls: 67 (8.6) years, 35.1% ♀ Ethnicity not stated	AD diagnosed by NINCDS-ADRD criteria. Assessment included MMSE, CDR, MRI, CTand PET	WPB	Pyrosequencing	<i>ALU</i> , <i>LINE-1</i> and <i>SAT-α</i>	AD decreased <i>ALU</i> (25.5% vs. 25.8, $P=0.1$), increased <i>LINE-1</i> (83.6% vs. 83.1, $P=0.2$). Only latter remained after adjustment. Sex differences observed: males slightly higher <i>ALU</i> , <i>LINE-1</i> and <i>SAT-α</i>	Age, sex
[54]	Case-control study. Samples from a geriatric neuropsychiatry DNA in Taipei, Taiwan	Cases with familial dementia excluded. Controls had no cognitive defects or sleep disorders	59 AD: 72.0 (9.7) years, 54% ♀ 14 DLB: 72.5 (9.3) years, 57% ♀ 7 other dementia: 60.8 (7.7) years, 43% ♀ 80 controls: 71.1 (9.4) years, 54% ♀ Ethnicity not stated	Probable AD diagnosed by NINCDS-ADRD criteria. Probable DLB diagnosed by criteria [†] . Assessment included MMSE and CDR	WPB	mPCR, ABI Prism 310 Genetic Analyzer	<i>PER1</i> , <i>PER2</i> , <i>PER3</i> , <i>CRY1</i> , <i>CRY2</i> , <i>CLOCK</i> , <i>BMAL1</i> , <i>ITIM</i> and <i>CK1ε</i>	DLB increased compared to AD ($P=0.007115$) and controls ($P=0.000365$)	None mentioned
[55]	Case-control study in Sao Paulo, Brazil. Recruitment details not provided	None	45 AD: 74.6 years (8.43) 50 older controls: 72.2 (8.2) years 50 younger controls: 21.1 (1.9) years Sex and ethnicity not stated	Probable AD diagnosed by NINCDS-ADRD criteria	WPB	mPCR, solid-phase DNA sequencing	<i>SIRT3</i> , <i>SMARCA5</i> , <i>HTERT</i> , and <i>CDHL</i>	HTERT hypomethylation in the elderly compared to young ($P=0.0024$) and AD ($P=0.0086$)	None mentioned
[56]	Case-control study. Recruitment details not provided	Cases had AD for at least 3.5 years	28 AD: 75.3 (7.9) years, 71.4% ♀ 28 older controls: 73.9 (8.1) years, 71.4% ♀ 28 young controls: 21.41 (0.9) years, 71.4% ♀ Ethnicity not stated	Probable AD diagnosed by NINCDS-ADRD criteria. Assessment included MMSE	WPB	Methylation-sensitive enzyme digestion	rDNA promoter, 18S and 28S	No difference	None mentioned
[57]	Betula 20-year longitudinal cohort recruited from register in Sweden.	Not diagnosed with dementia at baseline. Excluded individuals with	16 “maintainers”: 57.8 (3.6) years, 50% ♀	Dementia diagnosed	WPB	HM450K	353 CpG sites used to predict biological	DNAm age at FU was associated with dementia ($\beta = .16$, $P=0.019$)	Sex, chronological age, lifestyle, genetics,

Study	Recruitment and study design	Exclusion/Inclusion criteria	N, mean (SD) age, sex, ethnicity	Diagnosis	Tissue	Methylation platform	Gene region	DNA methylation finding	Adjustments
	Groups classified according to performance on repeat episodic memory test	cancer chemotherapy	20 "average": 58.0 (3.5 years) years, 55% ♀ 16 "decliners": 57.9 (3.6) years, 50% ♀ 0, 2, 9 with dementia, respectively. Ethnicity not stated				epigenetic (DNAm) age ^S		smoking, living situation, health
[58]	Case-control study, recruited from hospitals in Ehime, Japan	Controls without cognitive impairment or history of mental disorders	20 DLB: 77.8 (7.7) years, 55% ♀ 20 controls: 77.0 (6.9) years, 60% ♀ All Japanese	Probable DLB assessed with 2 core clinical features (fluctuating cognition, visual hallucinations, and/or parkinsonism)	PBL	Pyrosequencing	<i>SNCA</i> , 10 CpGs in intron	DLB decreased average (8.2 ± 2.6 vs. 10.5 ± 2.7 , $P < .001$) and at 1 site (5.1 ± 1.5 vs. 7.3 ± 2.9 , $P = .002$)	None mentioned
[59]	Case-control study. Sub-sample of participants from a larger study of neurodegeneration, USA	Controls without dementia or other neurological conditions	128 FTD: onset 58.7 (8.92) years, 47.4% ♀ 43 PSP: onset 63.4 (6.78) years, 28.0% ♀ 185 Controls: age not given, 62.4% ♀ >Caucasian .74%	Not mentioned.	WPB	HM450K	485,577 CpG sites	Differences and overlap between FTD and PSP. Most top DMPs located within 17q21.31 region, 4 in NFYA were hypomethylated	Batch effects, age, ethnicity, sex, blood cell counts
[60]	Case-control study, recruited from clinical units in Milan, Italy	Controls with no cognitive or psychobehavioral problems	38 FTLD: 67.45 (1.2 SEM) years, 44.7% ♀ 38 controls: 70.38 (1.4 SEM) years, 63.2% ♀ All Italian	FTLD diagnosed by criteria [¶] . Assessment included CDR, MMSE, FAB, WCST, ToL	WPB	MSP nPCR, MS Opticon 2 Light Cycler Instrument (Roche, Germany)	<i>GRN</i> promoter	FTLD increase (61.5 vs. 46.3 %, $P = .001$)	Age, sex
[61]	Case-control study, recruited from neurology departments and memory clinics in Belgium. Affected/at-risk relatives subsequently recruited.	All participants carried C9orf72 repeat expansion (G4C2 expansion) and MMSE >24. Controls had no neurodegenerative or psychiatric diseases	Sub-sample of the following participants (exact numbers and further details not given): 72 FTD or ALS cases, 61 at-risk relatives, 1044 unrelated controls Ethnicity not stated	FTD diagnosed by established [#] . ALS diagnosed by revised El Escorial criteria ^{**}	WPB	MSRE assays followed by qPCR	<i>C9orf72</i> : each G4C2 unit, 2 CpGs in 3' flanking region, 17 CpGs in 5' flanking CpG island	Methylation associated with genotype but no differences between case/control groups overall	None mentioned
[62]	Case-control, recruited from centers for neurodegenerative disease, USA	None	50 ALS: onset IQR 51.30–63.5 years, 40% ♀	ALS diagnosed by El Escorial criteria ^{**} . FTD diagnosed by criteria [#]	WPB	MSRE assays, followed by qPCR, Sanger sequencing	<i>C9orf72</i> promoter	No difference	Age at onset, age at death, disease duration, and

Study	Recruitment and study design	Exclusion/Inclusion criteria	N, mean (SD) age, sex, ethnicity	Diagnosis	Tissue	Methylation platform	Gene region	DNA methylation finding	Adjustments
[63]	Case-control study. ALS cases recruited from health clinic in Toronto, Canada, FTL D cases recruited from specialized hospitals	None mentioned. C9orf72 G4C2 repeat expansion carriers and noncarriers, both groups with and without dementia	33 FTD: onset IQR 50.73–62.0 years, 36% ♀ 19 controls: no details given Ethnicity not stated Carriers 35 ALS: 60.7 (8.5) years, 46% ♀ 25 FTL D: 62.6 (8.8) years, 48% ♀ 11 ALS-FTL D: 61.3 (6.9) years, 9% ♀ 9 controls: 71.2 (8.5) years, 67% ♀ Noncarriers: 54 ALS: 65.0 (8.2) years, 35% ♀ 59 FTL D: 74.3 (7.0) years, 56% ♀ 10 ALS-FTL D: 74.7 (10.3) years, 60% ♀ 76 controls: 73.0 (7.6) years, 63% ♀ Ethnicity not stated	ALS and FTL D diagnosed by criteria ^{**} ^{††} ^{‡‡}	WPB	ABI 3100 DNA Analyzer	C9orf72 G4C2 repeat methylation	No difference	None mentioned
[64]	Case-control study. Samples from the University of CNDR biorepository, USA	Carriers with C9orf72 mutation	22 carriers: 46–73 years, 36% ♀ (10 ALS, 6 ALSdementia, 6 FTL D) 16 non-carriers: 50–83 years, 44% ♀ (3 ALS, 3 AD, 1 each DLB, FTL D, VD, PD, 6 controls) Ethnicity not stated	Clinical FTL D features and neuropathological changes in carriers	WPB	MSRE assays,	C9orf72 promoter	Blood methylation data not presented (text states ~ 1/3 of carriers hypermethylated but not associated with clinical phenotype)	None mentioned
[65]	Case-control study of C9orf72, recruited from specialist hospitals. Location not specified	Controls > 62 years old. All participants unrelated. Carriers with C9orf72 mutation	34 carriers w/ FTL D: 63.0 (8.3) years, 53% ♀ 166 non-carriers w/ FTL D: 70.5 (8.1) years, 51% ♀ 103 controls: 73.2 (6.3) years, 55% ♀	FTL D diagnosed by criteria ^{††}	WPB	Sequencing chromatograms and mutation surveyor (Softgenetics)	C9orf72	FTL D increase in expansion carriers vs. noncarriers ($P=7.8E-13$) and controls ($P=8.3E-9$)	None mentioned

Study	Recruitment and study design	Exclusion/Inclusion criteria	N, mean (SD) age, sex, ethnicity	Diagnosis	Tissue	Methylation platform	Gene region	DNA methylation finding	Adjustments
[66]	Case report of 3 sibling pairs from a large kindred of European origins. No other info provided	Included HSN1 kindred	Most Italian (6 Argentinian, 4 Canadian) 3 pairs of HSN1 patients and their siblings. All Caucasian	DNMT1 heterozygous Y495 C mutation patients confirmed through medical records and genetic testing	PBBc	Whole genome sequencing, HiSeq 2000, Illumina	>1 billion 75-bp single-end reads	564,218 differentially methylated CpGs ($P < .05$) in paired sample analysis. Hypomethylation predominant in affected individuals	None mentioned
[67]	Case-control study, r4 HSN1 kindreds with sensorineural deafness and dementia recruited	None	12 HSN1 affected individuals: 18–59 years, 55% ♀ female, mix of European and non-European ancestry 12 genetically related unaffected (other details not given)	HSAN1 with dementia and hearing loss. Previous medical record	WPB	HM27K and pyrosequencing (DNMT1)	Global DNA methylation	Affected 8% reduction in global 5mC content ($P < .001$), increased methylation at 10 promoter CpGs (HM27 K), reduced <i>DNMT1</i> methylation in the satellite 2 region	None mentioned
[68]	Case report of a hospital patient with MAPT mutation G389 R in Shanghai, China. 3 years FU	Patient with mild atrophy of frontal lobe leading to FTD. Relatives had no similar clinical presentations	1 patient, 27 years, ♂ 2 unaffected carriers (family members) All Chinese	Rapid Progression FTD diagnosed with MoCA, MMSE, MRI, PET, blood and CSF measures	PBL	Pyrosequencing	<i>MAPT</i> ; 54 CpGs in the promoter/1st exon, 14 CpGs around the mutation site	No difference	None mentioned

Abbreviations: AD, Alzheimer's disease; ADAS, Alzheimer's Disease Assessment Scale—cognitive subscale; ADL, activity of daily living; ALS, amyotrophic lateral sclerosis; aMCI, amnesic mild cognitive impairment; *APOE*, apolipoprotein E; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; CDR, Clinical Dementia Rating; CNDR, Pennsylvania Center for Neurodegenerative Disease; CSF, cerebrospinal fluid; CT, computed tomography; DLB, dementia with Lewy bodies; DMR, differentially methylated region; DNAm, DNA methylation; DSM (III or IV), Diagnostic and Statistical Manual of Mental Disorders, 3rd and 4th revision; FAB, frontal assessment battery; FDG-PET, [18F]-fluorodeoxyglucose–positron emission tomography; FTD, frontotemporal dementia; FTLD, frontotemporal lobar degeneration; FU, follow-up; HM27K, Infinium HumanMethylation27 BeadChip; HM450K, Infinium HumanMethylation450 BeadChip; HSN1, hereditary sensory and autonomic neuropathy type 1 with dementia and hearing loss (HSAN1E; OMIM 614116); IADL, instrumental activity of daily living; ICD-10, International Statistical Classification of Diseases and Related Health Problems, 10th revision; LOAD, late-onset Alzheimer's disease; MADRS, Montgomery-Asberg Depression Rating Scale; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; MRC, Medical Research Council; MRI, magnetic resonance imaging; MS-HRM, methylation-sensitive high-resolution melting analysis; MSP, methylation-specific PCR; MSP rPCR, methylation-specific Primer Real Time; mPCR, methylation-specific PCR; MSRE, methylation-sensitive restriction enzyme; NDI, National Death Index; NINCDS-ADRD, National Institute of Neurological and Communicative Disorders and Stroke—Alzheimer's Disease and Related Disorders Association; NINDS-AIREN, National Institute of Neurological Disorders and Stroke—Association Internationale pour la Recherche et l'Enseignement en Neurosciences; NPI, Neuropsychiatric Inventory; PBBc, peripheral blood B cells; PB CD41 Tc, peripheral blood CD41 T cells; PBL, peripheral blood leukocytes; PBLy, peripheral blood lymphocytes; PBM, peripheral blood monocytes; PBMC, peripheral blood mononuclear cells; PSP, progressive supranuclear palsy; qMSP, quantitative methylation-specific PCR; ROS, Religious Orders Study; RMAP, Rush Memory Aging Project; ToL, Tower of London test; VD, vascular dementia; WBC, white blood cells; WBL, whole blood lymphocytes; WPB, whole peripheral blood; WCST, Wisconsin Card Sorting Test.

* Neary et al (1998) [69] and Rascovsky et al (2011) [70].

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- ⁷ Peterson et al (2001) [71] and McKhann et al (1984) [3].
- [‡]McKeith et al (1999) [72] and McKeith et al (1996) [73].
- [§]Horvath (2013) [14].
- [¶]Neary et al (1998) [69] and McKhann et al (2001) [74].
- [#]Gorno-Tempini et al (2011) [75] and Rascovsky et al (2011) [70].
- ^{**}Brooks et al (2000) [76].
- ^{7‡}Clinical and neuropathological criteria for frontotemporal dementia (1994) [77].
- ^{‡‡}Hughes et al (2001) [78].

Table 2

Studies of AD which measured DNA methylation using Infinium arrays

Study	Diagnosis	Tissue	(n)	Method	Finding
[57]	Cognitive decline/dementia	WPB	52	HM450K	DNAm age at follow-up associated with dementia ($\beta = 0.16$, $P = .019$)
[29]	AD	WPB	57	HM450K	805 differentially methylated CpGs.
[41]	AD	WPB	57	HM450K	AD associated with differential methylation at <i>DAPK1</i> , <i>GAS1</i> , <i>NDUFS5</i>
[28]	AD, aMCI	WBC	12	HM450K	1021 loci different between 3 groups, 156 associated with symptom progression.
[33]	AD	PB CD4+ Tc	15	HM450K	Small differences in global methylation (<5%).
[30]	AD, MCI	WPB	128	HM27K	AD lower <i>HMOX1</i> methylation (77.94 ± 13.0 vs. controls 87.20 ± 3.1).

Abbreviations: AD, Alzheimer’s disease; aMCI, amnesic mild cognitive impairment; DNAm, DNA methylation; HM450K, InfiniumHumanMethylation450 BeadChip; HM27K, Infinium HumanMethylation27 BeadChip; PB CD41 Tc, Peripheral blood CD41 T cells; WBC, white blood cells; WPB, whole peripheral blood.

Table 3

Candidate genes examined in more than one AD study

Study	Gene	(n)	Methylation method	DNA methylation association
[22]	<i>APOE</i>	2	MSP rtPCR	None
[46]		135	mPCR	None
[47]		15	Pyrosequencing	None
[22]	<i>APP</i>	2	MSP rtPCR	Increase (4.1% vs. 3.6%), no <i>P</i> value given
[46]		135	MSP	Decrease, no effect given (<i>P</i> < .05)
[27]	<i>BACE1</i>	200	MS-HRM	Nonsignificant increase (0.5 ± 0.9 vs. 0.8 ± 1.2 , <i>P</i> = .06)
[37]		135	MS-HRM	Nonsignificant increase (0.62 ± 0.11 vs. 0.74 ± 0.11 , <i>P</i> = .12)
[34]	<i>BDNF</i>	40	MSP rtPCR	None
[38]		106	Pyrosequencing	Increased mean (9.50 ± 4.43 vs. 7.45 ± 2.77 , <i>P</i> = .004) and at all CpGs
[36]		40	ABI 3730 DNA analyzer	Increase ($5.08 \pm 5.52\%$ vs. $2.09 \pm 0.81\%$, <i>P</i> = .041) and at 1 site (4.85 ± 6.91 vs. 0 ± 0 , <i>P</i> = .014)
[27]	<i>DNMT1</i>	200	MS-HRM	None
[37]		135	MS-HRM	None
[27]	<i>DNMT3A</i>	200	MS-HRM	None
[37]		135	MS-HRM	Nonsignificant decrease (0.90 ± 0.25 vs. 1.59 ± 0.26 , <i>P</i> = .10).
[27]	<i>DNMT3B</i>	200	MS-HRM	None
[37]		135	MS-HRM	None
[53]	<i>LINE-1</i>	81	Pyrosequencing	Increase (83.6% vs. 83.1%, <i>P</i> = .02).
[39]		58	MS-HRM	None
[21]	<i>MTHFR</i>	14	22 gene array	None
[27]		200	MS-HRM	None
[37]		135	MS-HRM	None
[48]	<i>PIN1</i>	60	MSP rtPCR	Decrease (3.76 ± 0.89 vs. 11.45 ± 2.26 , <i>P</i> = .001).
[22]		2	MSP rtPCR	Decrease (0.9% vs. 2.9%), no <i>P</i> value given
[26]		317	Pyrosequencing	AD, none. FTD increase compared to AD and controls (<0.4%, <i>P</i> < .03)
[34]	<i>PSEN1</i>	40	MSP rtPCR	None
[21]		14	22 gene array	None
[22]		2	MSP rtPCR	None
[27]		200	MS-HRM	Nonsignificant decrease (0.6 ± 1.2 vs. 0.9 ± 1.9 , <i>P</i> = .18).

Study	Gene	(n)	Methylation method	DNA methylation association
[37]		135	MS-HRM	None
[34]	<i>SIRT1</i>	40	MSP-rPCR	None
[22]		2	MSP-rPCR	None
[50]		84	Sequenom EpiTYPER	None
[46]		135	MSP	Hypermethylation (no effect size/ <i>P</i> value given)
[45]	<i>Sub-telomeric DNA</i>	52	MspI and HpaII telomere	None
[52]		99	length distributions	Faster telomeric hypomethylation with aging (0.0435 vs. 0.0340, no <i>P</i> value given).

Abbreviations: *APOE*, apolipoprotein E; FTD, frontotemporal dementia; MSP rPCR, methylation-specific primer real time PCR; mPCR, methylation-specific high-resolution melting analysis.