BLOOD-BASED BIOMARKERS

Blood DNA methylation signatures to detect dementia prior to overt clinical symptoms

Peter Daniel Fransquet1 | Paul Lacaze1 | Richard Saffery2 | James Phung1 | Emily Parker1 | Raj Shah3 | Anne Murray4 | Robyn L. Woods1 | Joanne Ryan1,5

1 School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia
2 Department of Paediatrics, Murdoch Children’s Research Institute, The University of Melbourne, Parkville, Victoria, Australia
3 Department of Family Medicine and Rush Alzheimer’s Disease Center, Rush University Medical Center, Chicago, Illinois, USA
4 Berman Center for Outcomes and Clinical Research, Hennepin Healthcare, Division of Geriatrics, Department of Medicine, Hennepin Healthcare Research Institute, University of Minnesota, Minneapolis, Minnesota, USA
5 PSNREC, Université de Montpellier, INSERM, Montpellier, France

Correspondence
Dr. Joanne Ryan, Monash University, The Alfred Hospital, 55 Commercial Road, Melbourne 3004, Australia. E-mail: joanne.ryan@monash.edu

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Abstract

Introduction: This study determined whether blood DNA methylation (DNAm) patterns differentiate individuals with presymptomatic dementia compared to controls.

Methods: DNAm was measured in 73 individuals prior to dementia diagnosis and 87 cognitively healthy controls matched for age, sex, smoking, education, and baseline cognition. DNAm was also measured at 3 years follow-up in 25 dementia cases, and 24 controls.

Results: Cases and controls differed in DNAm (unadjusted \( P < .01 \)) at the time of diagnosis (\( n = 28,787 \) probes), and pre-diagnosis (\( n = 15,111 \) probes), with cg01404610 (General transcription factor IIA subunit 1 gene) significant after correction for multiple testing. Overall, 1150 probes overlapped between analyses (methylation differences from –10.6% to +11.0%), and effect sizes increased from pre-diagnosis to diagnosis.

Discussion: Discernible blood DNAm signatures are in dementia cases before the appearance of overt clinical symptoms. Blood-based methylation may serve as a potential biomarker of dementia, but further investigation is needed to determine their true clinical utility.

KEYWORDS biomarker, dementia, epigenome-wide association study, methylation, pre-diagnosis

1 INTRODUCTION

Dementia is a neurodegenerative disease with no cure, preventive intervention, or treatment to slow onset. It involves a loss of cognitive abilities, primarily memory loss, that impacts an individual’s independence in day-to-day functioning.\(^1\) An early dementia diagnosis is difficult to obtain due to the often gradual onset in symptoms that are nonspecific and complex.\(^2\) As a result, a dementia diagnosis is often made in the late stages of the disease, after a multitude of visits to general practitioners and specialists for comprehensive cognitive...
screening, blood tests, analysis of cerebrospinal fluid constituents, and brain imaging, and by ruling out other underlying conditions which can present with overlapping symptoms. Further, a definitive diagnosis of the most common cause of dementia, Alzheimer’s disease (AD), can only be determined from an autopsy.

Objective biomarkers for dementia are severely lacking. Identification of definitive diagnostic biomarkers would permit an earlier diagnosis, which would allow for individuals diagnosed to make decisions about their future (eg, medical, legal, and financial) while they still have the cognitive capacity. Accurate biomarkers of dementia in the preclinical, asymptomatic stage may be crucial to the development of effective treatment and prevention strategies. They would facilitate effective recruitment into clinical trials at an earlier time-point of the disease, which could then lead to early disease interventions.

There is increasing interest in the potential of epigenetic biomarkers in blood to facilitate early diagnosis of disease. Epigenetics refers to molecular modification of DNA that can regulate gene expression without altering the DNA sequence. Epigenetic mechanisms have been implicated in the etiology of dementia. Recent evidence suggests that DNA methylation (DNAm) signatures may also be detected in blood that differentiates individuals with dementia compared to those without. However, at present the degree to which blood-based DNAm patterns have prognostic utility in dementia, prior to the appearance of clinical symptoms, remains unclear.

This study investigated whether DNAm differences could be identified in the blood of individuals several years prior to a dementia diagnosis (cases), compared to individuals who remain cognitively healthy (controls). Second, this study investigated whether these same DNAm patterns differentiated individuals with dementia at the time of diagnosis and without.

2 METHODS

2.1 Study sample

Data and biospecimens for this study came from the Aspirin in Reducing Events in the Elderly (ASPREE) cohort, which has been detailed previously. In brief, ASPREE is a randomized, double-blind, placebo-controlled study of the effects of low-dose aspirin on disability-free survival in 19,114 older community-dwelling individuals. Participants were recruited from general practice (in Australia, n = 16,703) or clinical trial networks (in the United States, n = 2411). Exclusion criteria included a previous dementia diagnosis, or a score of < 78 on the Modified Mini-Mental State Examination (3MS), which excluded severe cognitive impairments. The study was in accordance with the Declaration of Helsinki 2008 revision, the National Health and Medical Research Council (NHMRC) Guidelines on Human Experimentation, the federal patient privacy (Health Insurance Portability and Accountability Act [HIPAA]) law, the International Conference for Harmonisation Guidelines for Good Clinical Practice, and the Code of Federal Regulations. The trial ran for a median of 4.7 years. Neurocognitive assessment was conducted in participants at baseline, 1-year, and 3-year follow-up time points. These included the 3MS, a modified version of the Mini-Mental State Examination which assesses global cognitive function, with a score of less than 78 out of 100 indicative of cognitive impairments; the Symbol Digit Modalities Test (SDMT), a timed test of attention and processing speed; the Controlled Oral Word Association Test (COWAT) using the letter F, which assess language/verbal fluency by asking participants to name as many words starting the letter “F” in a given time frame; and the Hopkins Verbal Learning Test Revised (HVLT-R) to measure delayed recall memory on a scale of 0 to 12, with higher scores indicating better test performance.
2.2 Dementia diagnosis in Aspirin in Reducing Events in the Elderly (ASPREE)

Over the trial, individuals with a score of < 78 on the 3MS, a drop of > 10.15 on the 3MS, who commenced dementia medication, or who had a dementia diagnosis, were considered possible dementia cases. These individuals underwent additional cognitive assessments and physical function questionnaires. An international panel of clinical specialists, including neurologists, neuropsychologists, and geriatricians from Australia and the United States reviewed the cognitive and functional assessments, medical records, and clinical diagnosis information (as well as the results of blood tests and brain scans when available) to reach a diagnosis consensus based on Diagnostic and Statistical Manual for Mental Disorders, American Psychiatric Association (DSM-IV) criteria. From the 19,114 participants, 964 triggered further assessments for dementia, and 575 individuals were adjudicated as having dementia by the end of the trial period.

2.3 Selected subsample

This substudy consisted of 160 Australian-based participants. Dementia cases and cognitively healthy controls were selected from individuals who provided blood samples at study randomization (baseline) when all individuals were cognitively healthy, and based on their dementia status at the year 3 follow-up. Controls were required to be without a dementia diagnosis over the duration of the trial, while dementia cases needed to have triggered further dementia assessment (which resulted in subsequent adjudication as dementia) at least 1 year after baseline and within 9 months of providing their 3-year blood sample. The cases (n = 73) and controls (n = 87) were additionally matched on age, self-reported sex, education, smoking status, and cognitive function at baseline. Forty-nine participants who provided samples at inclusion had also provided samples at follow-up (25 cases and 24 controls), and were included in the follow-up analysis (diagnosed dementia vs controls).

2.4 Quality control and methylation profiling

DNA from peripheral blood was extracted using Qiagen DNeasy Blood & Tissue Kits. Cytosine-phosphate-guanine (CpG) probe methylation was measured using the Illumina Infinium MethylationEPIC BeadChip (EPIC) at the Australian Genome Research Facility, Melbourne Victoria (http://www.agrf.org.au/).

All data were analyzed using R version 3.5.1 following an adapted Bioconductor workflow protocol. Quality control found no failed genomic position probes, defined as both the methylated and unmethylated channels reporting background signal levels (ie, no probe detected), to a significance level of $P < .002$ (“detection,” R package Minfi). Sample data were normalized using subset quantile normalization (“preprocessQuantile”). Self-reported sex was confirmed as concordant with biological sex using “getSex” of the minfi R package.

Probes that failed in at least one sample, with known single nucleotide polymorphisms, sex chromosome probes, and cross-reactive probes, were removed from the data set (Table S1 in supporting information). A total of 626,793 probes were available for further analysis. All probes are mapped to the human genome assembly GRCh37 (hg19). Data were transformed into “M values” (log2 of array intensities at each probe) and “Beta values” (average DNAm within a sample as a measure between zero (probe is unmethylated) and one (100% methylated)).

2.5 Statistical analysis

Analysis was undertaken for both pre-diagnosis “cases” versus “controls” (baseline blood samples) and dementia cases versus controls (follow-up blood samples).

A principal component analysis was used to determine the degree to which technical or environmental variables influenced variability of methylation in the data set (“WGCNA”, Figures S1–S4 in supporting information). Differentially methylated probes were obtained using R package “limma” linear regression models, adjusting for possible confounding variables. Models included dementia status, age, sex, and methylation measurement batch in both analyses. Observations were adjusted for false discovery rate by the Benjamini and Hochberg (BH) method.

Stats 15 was used for probe wise t-tests between cases and controls, using methylation beta values from top probes in aforementioned models (ie, highest significance, or greatest change in methylation in cases compared to controls). Regression analysis was carried out to determine whether differences seen remained after adjusting for common confounding factors, being age, sex, and methylation measurement batch. Probe nomenclature is labelled as the Illumina EPIC probe name, followed by the methylated dinucleotide, chromosome, and genomic location (bp), for example, cg01404610, CpG14:81687455.

As the aim of this study’s analyses was discovery, further analysis took into account all probes which reached non-BH adjusted $P$-value of $< .01$, chosen as a conservative cut-off compared to $P < .05$. Differentially methylated region (DMR) analysis was carried out using “DMRcate” to identify gene regions that contained multiple significant probes. DMRcate ranks DMRs by Stouffer’s test statistic, which is used to combine the results from several independent tests bearing upon the same overall hypothesis. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases were used in gene pathway analysis, carried out using the “gometh” function from the missMethyl R package.

3 RESULTS

3.1 Baseline participant characteristics

Baseline characteristics of the 160 participants, 73 pre-diagnosis cases, and 87 controls are shown in Table 1. Only average COWAT scores differed between the two groups.
### TABLE 1  Baseline characteristics of participants (n = 160)

| Participant characteristics | Controls (n = 87) | Pre-diagnosed Dementia (n = 73) | P |
|----------------------------|------------------|---------------------------------|---|
| Age, mean, (SD)            | 76.4 (4.6)       | 77.6 (5.1)                      | 0.11 |
| Sex, n (%)                 | 50 (57.5)        | 42 (57.5)                       | 0.99 |
| Years to incident dementia mean (SD) | – | 3.7 (1.1)          | NA |
| Smoking                    |                  |                                 |     |
| Current                    | 2 (2.3)          | 0 (0)                           | 0.42 |
| Past                       | 36 (41.4)        | 32 (43.8)                       |     |
| Never                      | 49 (56.3)        | 41 (56.2)                       |     |
| Education                  |                  |                                 |     |
| <= 12 years                | 60 (69)          | 43 (58.9)                       | 0.19 |
| > 12 years                 | 27 (31)          | 30 (41.1)                       |     |
| 3MS, mean, (SD)            | 93.2 (4.8)       | 92.1 (4.4)                      | 0.14 |
| SDMT, mean, (SD)           | 36.2 (9.1)       | 35.0 (8.4)                      | 0.39 |
| COWAT                      | 12.8 (3.6)       | 14.1 (4.0)                      | 0.03 |
| HVLT-R delayed recall†    | 8.2 (1.9)        | 7.8 (1.9)                       | 0.2 |

Abbreviations: 3MS, Modified Mini-Mental State Examination; COWAT, Controlled Oral Word Association Test; HVLT-R, Hopkins verbal learning Test Revised; SD, standard deviation; SDMT, Symbol Digit Modalities Test. *Data not available for one participant.

The median time between baseline and the follow-up visits was 2.98 years. At follow-up, 49 of the aforementioned participants, 24 controls, and 25 cases with incident dementia also provided blood samples for DNA methylation analysis (Figure S5 and Table S2 in supporting information). Education level differed between dementia cases and controls (P = .005). Cases also had significantly lower scores on the 3MS, SDMT, and HVLT-R delayed recall.

#### 3.2 Differentially methylated probes between dementia cases prior to diagnosis and controls

A model adjusting for age, sex, and methylation batch showed one probe that passed BH adjustment for multiple testing (cg01404610, CpG14:81687455, Δ = +0.87%, BH Adj.P = .018, Figure 1a). This probe is situated between the long intergenic non-protein coding RNA gene. Other top probes ranked by higher and lower effect sizes (P < .05) can be seen in Table S3 and Table S4 in supporting information, respectively.

#### 3.3 Differentially methylated probes between dementia cases at diagnosis and controls

When comparing dementia cases versus controls, no probes passed BH adjustment for multiple testing. When adjusting for age, sex, and methylation batch only, a total of 28,787 probes (4.6% of total probes) were found to be differentially methylated between controls and those with a dementia diagnosis (P < .01), with the majority (14,879, 57.7%) being lower in methylation compared to controls. Top probes ranked by P-value, adjusted for age, sex, and batch, can be seen in Table 3, and top probes ranked by effect size (P < .05) can be seen in Table S5 in supporting information (higher methylation) and six lower methylation.

The largest methylation difference was a lower methylation at cg08642068, CpG20:31591776, Δ = -15.27%, SE: 4.37%, P = 0.001, Adj.P = 0.001 (Figure 1f, Table S6 in supporting information). This probe is within the Sad1 and UNC84 domain containing 5 (SUN5) gene.

#### 3.4 Common differentially methylated probes between pre-diagnosis and diagnosed dementia analyses

There were 1150 differentially methylated probes (DMPs) common between the two analyses, when adjusting for age, sex, and batch (P < .01). The top 10 common probes, ranked by significance level in both analyses, can be seen in Table S7 in supporting information. In all cases, the direction of association was the same, with a generally larger effect size seen when comparing cases and controls at diagnosis, compared to pre-diagnosed cases and controls. Of these, the largest methylation difference was a lower methylation in dementia diagnosis compared to controls, at cg06354780, CpG1:77296123, (-9.13%, SE:1.13%, P < .0001); prior to dementia diagnosis this difference was less than half (-3.41%, SE:1.21%, P = .005). This probe is situated between the long intergenic non-protein coding RNA 2567 (LINC02567), and ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 5 (ST6GALNAC5) genes.

#### 3.5 Differentially methylated regions

Regions of the genome which were found to be differentially methylated between cases and controls, differentially methylated regions (DMRs), were defined if they included at least three probes that were significantly different (P < .01) within the above DMP analyses, and had an average effect size of at least 0.5% methylation difference across the region. At baseline, there were 377 DMRs associated with pre-diagnosed dementia, which contained at least one probe with a minimum false discovery rate (FDR) of P < .01 (File S1 in supporting information).
### Pre-diagnosed dementia analysis

**Figure 1** Top differential probes comparing methylation of pre-diagnosed dementia (n = 73) versus controls (n = 87; a–e), and diagnosed dementia (n = 25) versus controls (n = 25; f–j). a, Probe CpG14:81687455, cg01404610 was the only probe to pass Benjamini and Hochberg adjustment for multiple testing (Δ –0.87%, SE:0.15, \(P < .0001\)). b, CpG8:49427283, cg03635442, Δ –7.89%, SE:2.97, \(P = .009\). c, CpG13:108739184, cg07674804, Δ +6.46%, SE:1.75, \(P = .002\). d, CpG17:154499, cg10440639, Δ –6.40%, SE:1.73, \(P = .0003\). e, CpG12:63696410, cg 04928577, Δ +6.11%, SE:1.75, \(P = .0006\). f, CpG20:31591776, cg08642068, Δ –15.27%, SE:4.37, \(P = .001\). g, CpG2:131058184, cg01311063, Δ –14.96%, SE:6.33, \(P = .02\). h, CpG19:44645078, cg23489630, Δ –14.61%, SE:3.49, \(P = .0001\). i, CpG22:45809952, cg01808030, Δ +11.71%, SE:4.61, \(P = .02\). j, CpG17:5403337, cg15954353, Δ +11.36%, SE:3.48, \(P = .002\). * \(P < .05\), ** \(P < .01\), *** \(P < .001\), **** \(P \leq .0001\)

Comparisons of DMRs between analyses were made using the DMR’s exact genomic coordinates, resulting in 21 DMRs common between pre-diagnosed and diagnosed dementia (Table 4). All common DMRs had the same direction of association between baseline and follow-up, with all but two being lower in the same direction over time (HOXA7 average methylation difference –1.05% at baseline but –0.92% at follow-up, and ADAM12 average methylation difference –2.16% at baseline but –1.90% at follow-up).

#### 3.6 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis

Pathways associated with pre-diagnosed dementia and dementia cases can be seen in Table 5. Pre-diagnosed dementia was only associated with one pathway from each database when adjusting for FDR, GO:0005654, nucleoplasm (1438/3123 methylated genes in information). These DMRs included between 3 and 21 methylation probes, and covered regions between 7 and 2461 base pairs (bp) long. However, none of these DMRs passed adjustment for multiple testing. The DMR with the largest average methylation difference was at vault RNA 2-1 (VTRNA2-1), from 7 probes within 384 bp region (Δ = –4.53%, Stouffer Adj. \(P = .52\)).

At follow-up, there were 1906 DMRs associated with dementia which contained at least one probe with a minimum FDR of \(P < .01\). These DMRs included between 3 and 56 probes, and regions of 12 to 3783 bp in length (File S1). Eighty-four DMRs passed Stouffer adjustment (\(P < .05\)). The most significant DMR overlapped the integrin subunit alpha E (ITGAE) and nuclear cap binding dudunit 3 (NCBP3) gene regions (13 probes in a 2894 bp region, chr17:3,704,471-3,707,364, Δ = –4.48%, Stouffer Adj. \(P = .005\)). The largest average methylation difference was seen in a CpG island preceding the kinesin family member 25 antisense RNA 1 (KIF25-AS1) gene (5 probes in a 269 bp region, chr6:168,393,930-168,394,198, Δ = –6.32%, Stouffer Adj. \(P = .038\)).
TABLE 2  Top 10 differentially methylated probes ranked by significance *, comparing baseline blood sample methylation between pre-diagnosis dementia cases (n = 73) and controls (n = 87)

| CpG Unit | Genomic location | Gene context | Pre- diagnosis dementia | μ (%) | Δ | SE | P       |
|----------|------------------|--------------|-------------------------|-------|---|----|--------|
| cg01404610 | Chr14:81687455   | GTF2A1       | No                      | 3.74% | +0.87% | 0.15% | <.0001 |
|          |                  |              | Yes                     | 4.61% |     |    |        |
| cg04298511 | Chr7:70159330    | AUTS2        | No                      | 5.26% | −1.33% | 0.31% | <.0001 |
|          |                  |              | Yes                     | 3.93% |     |    |        |
| cg12679980 | Chr5:43557583    | <PAIP1 > NNT-AS1 | No                  | 15.01% | +2.59% | 0.48% | <.0001 |
|          |                  |              | Yes                     | 17.61% |     |    |        |
| cg16274893 | Chr14:69204080   | <RAD51B > ZFP36L1 | No                   | 73.48% | −1.82% | 0.36% | <.0001 |
|          |                  |              | Yes                     | 71.66% |     |    |        |
| cg22871559 | Chr1:206730254   | RASSF5       | No                      | 3.53% | +0.43% | 0.09% | <.0001 |
|          |                  |              | Yes                     | 3.96% |     |    |        |
| cg19217964 | Chr16:63402222   | <CDH8 > CDH11 | No                      | 57.12% | +2.34% | 0.48% | <.0001 |
|          |                  |              | Yes                     | 59.46% |     |    |        |
| cg13616097 | Chr22:46372744   | WNT7B        | No                      | 9.36% | −2.33% | 0.53% | <.0001 |
|          |                  |              | Yes                     | 7.03% |     |    |        |
| cg02608511 | Chr8:71135222    | NCOA2        | No                      | 78.51% | −1.84% | 0.37% | <.0001 |
|          |                  |              | Yes                     | 76.67% |     |    |        |
| cg14609448 | Chr21:34896882   | GART         | No                      | 85.93% | −1.53% | 0.31% | <.0001 |
|          |                  |              | Yes                     | 84.40% |     |    |        |
| cg09251344 | Chr15:67814243   | C15orf61     | No                      | 3.82% | +0.43% | 0.09% | <.0001 |
|          |                  |              | Yes                     | 4.25% |     |    |        |

Note: These results remained significant after adjustment for age, sex, and methylation processing batch.

*Ranked by limma analysis significance level, μ, mean methylation %; Δ, mean methylation difference.

Gene context “>” represents gene downstream from probe, “<” represents gene upstream from probe, otherwise probe sits within the gene.

pathway, FDR Adj. P = .001), and hsa:04360, Axon guidance (114/181 methylated genes in pathway, FDR Adj. P = .02). At follow-up, there were three pathways associated with dementia using GO and 15 using KEGG. The top dementia associated KEGG pathway was also hsa:04360 Axon guidance (133/181 methylated genes in pathway, FDR Adj. P = .001).

4  | DISCUSSION

Given that approximately 60% of dementia cases go undiagnosed, it is clear that there is an urgent need for definitive biomarkers of the disease. Here we have identified DNAm signatures in the blood of apparently healthy older individuals, which differentiate those individuals who remain cognitively healthy from those who are diagnosed with dementia 3 years later. Some probes, gene regions, and pathways that we identified appear biologically relevant, suggesting that these may not only serve as biomarkers, but may potentially be involved in the pathogenesis of the disease. Further, the consistency in many of the main findings, and the increasing effect size between the analysis of pre-diagnosed and diagnosis DNAm profiles, suggests a potential relationship between these DNAm signatures and the appearance of disease symptoms and thus, progression. Only one probe (cg01404610) passed adjustment for multiple testing in pre-diagnosis analysis. It is within the GTF2A1 gene, a part of the TFIID-DNA complex which is involved in transcription initiation. This is a novel finding, and the exact role in dementia or biological processes disrupted in dementia, remains unclear.

Despite not reaching significance after correction for multiple testing, there were several other individual probes in gene regions previously implicated in dementia. For example, cg1647496, CpG19:13875014 (Δ +5.99%, pre-diagnosis cases), is within a CpG island upstream from methylthioribose-1-phosphate isomerase 1 (MRI1) gene. MRI1 is an enzyme involved in the methionine salvage pathway, which results in the formation of the amino acid methionine via reprocessing cellular metabolites that contain sulphur. A previous study (n = 11) also showed increased methylation in the same MRI1 region at a nearby probe (only 98 bp away) in the brain (Δ +25.8%) and blood (Δ +28.4%; cg25755428) to be associated with Parkinson’s disease. Further details of findings which align with previously identified genes involved in dementia are given in Appendix S1 in supporting information.

Additionally, some of the common DMRs are in the regions of genes which have previously been implicated in key pathological processes of dementia. Amyloid beta (Aβ) build-up is one of the hallmark pathologies of AD, and thought to be present prior to clinical manifestation
TABLE 3

Top 10 differentially methylated probes ranked by significance, comparing follow-up blood sample methylation between diagnosed dementia cases (n = 25) and controls (n = 24)

| CpG Unit | Genomic location | Gene context | Dementia | µ (%) | Δ | SE | P |
|----------|------------------|--------------|----------|-------|---|----|---|
| cg17750831 | chr3: 123304350 | <HACD2> MYLK-AS1 | No | 2.92% | +0.65% | 0.10% | <.0001 |
| cg13153264 | chr1: 152680502 | <LCE2A> LCE4A | No | 51.73% | −7.12% | 1.20% | <.0001 |
| cg15197125 | chr8: 128859546 | PVT1/MYC | No | 53.62% | −6.28% | 1.07% | <.0001 |
| cg14350179 | chr22: 43411101 | PACSIN2 | No | 3.24% | +0.75% | 0.14% | <.0001 |
| cg06823517 | chr5: 35484879 | <PRLR > SPEF2 | No | 79.93% | −3.55% | 0.62% | <.0001 |
| cg26390944 | chr13: 78433828 | EDNRB-AS1 | No | 66.32% | −5.19% | 0.95% | <.0001 |
| cg05868469 | chr15: 93128777 | <LINC00930 > FAM174B | No | 59.28% | +4.77% | 0.85% | <.0001 |
| cg22455795 | chr19: 41109651 | LTBP4 | No | 20.67% | +6.78% | 1.45% | <.0001 |
| cg10491563 | chr3: 158378015 | GFM1 | No | 53.43% | −11.35% | 2.28% | <.0001 |
| cg03300589 | chr5: 41510820 | <PLCXD3> QXCT1 | No | 17.65% | −3.32% | 0.69% | <.0001 |

Note: These results remained significant after adjustment for age, sex, and methylation processing batch.

* Ranked by limma analysis significance level, µ, mean methylation %; Δ, mean methylation difference.

Gene context “>” represents gene downstream from probe, “<” represents gene upstream from probe, otherwise probe sits within the gene.

The main strength of this study is the comprehensive, in-depth cognitive testing available on all participants from baseline to follow-up, including adjudicated dementia diagnosis. The cognitive screening at baseline ensured that all participants were cognitively healthy at study entry, when the first blood sample was collected. The longitudinal nature of the study with the collection of blood samples at two time-points (both prior to and at the time of dementia diagnosis) meant we were able to not only carry out a dementia case control analysis, but also compare methylation profiles of cases and controls prior to appearance of clinical symptoms. This provided a unique opportunity to generate pre-clinical methylation profiles and to identify genes that are possibly involved in the progression of dementia. Most other
disease, and the leading cause of blindness, but they are also a known risk factor for cognitive decline and dementia. KEGG:hsa04724, is involved in glutamatergic synapse function, and may also be involved in the pathology of dementia. This pathway was found to be differentially methylated only in the diagnosed dementia group (81 of 114 genes, FDR Adj.P = .046). Glutamatergic signaling occurs in synapses throughout the nervous system, and is involved in neuronal excitability and neurotransmission.41 Because of this it is seen as a potential drug target for neurological disorders including dementias.41 Another study has also recently observed this pathway to have differential expression in association with AD.42
| Gene   | Genomic location       | DMR length (bp) | Number of probes | Time point | Stouffer | Mean DMR Δ % |
|--------|------------------------|-----------------|------------------|------------|----------|---------------|
| GULP1  | chr2:189156425-189157566 | 1142            | 15               | Baseline   | 0.45     | −0.89         |
|        |                        |                 |                  | Follow-up  | 0.04     | −1.42         |
| SORCS3 | chr10:106400565-106401517 | 953             | 6                | Baseline   | 0.54     | −1.26         |
|        |                        |                 |                  | Follow-up  | 0.42     | −1.88         |
| PIEZO2 | chr18:11148510-11149470 | 961             | 10               | Baseline   | 0.51     | −1.56         |
|        |                        |                 |                  | Follow-up  | 0.80     | −1.94         |
| DNAH14 | chr1:225117076-225117676 | 601             | 7                | Baseline   | 0.64     | −1.61         |
|        |                        |                 |                  | Follow-up  | 0.23     | −2.65         |
| RIBC2  | chr22:45809244-45809952 | 709             | 13               | Baseline   | 0.68     | 1.68          |
|        |                        |                 |                  | Follow-up  | 0.43     | 2.99          |
| FOXG1  | chr14:29235904-29236535 | 632             | 13               | Baseline   | 0.62     | −1.26         |
|        |                        |                 |                  | Follow-up  | 0.21     | −1.81         |
| HOXC5  | chr12:54425156-54425634 | 479             | 3                | Baseline   | 0.32     | −1.87         |
|        |                        |                 |                  | Follow-up  | 0.19     | −2.46         |
| EPHA6  | chr3:96532859-96533824  | 966             | 8                | Baseline   | 0.44     | −1.44         |
|        |                        |                 |                  | Follow-up  | 0.04     | −2.71         |
| HOXA7  | chr7:27195602-27196153  | 552             | 7                | Baseline   | 0.53     | −1.05         |
|        |                        |                 |                  | Follow-up  | 0.29     | −0.92         |
| SLC6A2 | chr16:55689851-55690418 | 568             | 10               | Baseline   | 0.72     | −1.02         |
|        |                        |                 |                  | Follow-up  | 0.61     | −1.29         |
| MIR199A2| chr1:172113506-172114419 | 914              | 9                | Baseline   | 0.64     | −0.97         |
|        |                        |                 |                  | Follow-up  | 0.31     | −1.46         |
| SYN3   | chr22:33453893-33454632 | 740             | 10               | Baseline   | 0.82     | −0.66         |
|        |                        |                 |                  | Follow-up  | 0.18     | −2.51         |
| SLC4A10| chr2:162283705-162284206 | 502             | 6                | Baseline   | 0.60     | −0.88         |
|        |                        |                 |                  | Follow-up  | 0.43     | −1.36         |
| IRX4   | chr5:1886956-1887583    | 628             | 14               | Baseline   | 0.87     | −0.98         |
|        |                        |                 |                  | Follow-up  | 0.73     | −1.70         |
| CNTN1  | chr12:41221505-41221855 | 351             | 7                | Baseline   | 0.58     | 1.98          |
|        |                        |                 |                  | Follow-up  | 0.03     | 4.65          |
| ARRDC4 | chr15:98503768-98503878 | 111             | 7                | Baseline   | 0.46     | −0.69         |
|        |                        |                 |                  | Follow-up  | 0.32     | −1.04         |
| NOS1   | chr12:117798627-117799083 | 457             | 3                | Baseline   | 0.46     | −1.10         |
|        |                        |                 |                  | Follow-up  | 0.19     | −2.59         |
| LOC101929268| chr8:49468684-49469113 | 430             | 8                | Baseline   | 0.54     | −0.91         |
|        |                        |                 |                  | Follow-up  | 0.21     | −1.41         |
| EDIL3  | chr5:83680045-83680326  | 282             | 7                | Baseline   | 0.52     | −1.38         |
|        |                        |                 |                  | Follow-up  | 0.23     | −2.29         |
| MARCH3 | chr5:126205009-126205081 | 73              | 3                | Baseline   | 0.37     | −2.03         |
|        |                        |                 |                  | Follow-up  | 0.16     | −2.69         |
| ADAM12 | chr10:128076910-128076941 | 32              | 3                | Baseline   | 0.38     | −2.16         |
|        |                        |                 |                  | Follow-up  | 0.47     | −1.90         |

Abbreviations: Bp, basepairs; DMR, differentially methylated region.

*Common DMR is defined here as 21 regions showing the exact genomic location in both pre- and post-diagnosis blood sample analyses.
TABLE 5  Gene pathway analysis using top methylated probes

| Database | Time point | Pathway | Term | Number of genes in pathway | Number of differentially methylated genes | P   | FDR  |
|----------|------------|---------|------|----------------------------|------------------------------------------|-----|-------|
| GO       | Pre-diagnosis | GO:0005654 | Nucleoplasm | 3123 | 1438 | 6.37e-08 | 0.001 |
|          | Diagnosis    | GO:0007165 | Signal transduction | 827  | 491  | 4.16e-08 | 0.0007 |
|          |             | GO:0003779 | Actin binding | 236  | 165  | 9.98e-07 | 0.007 |
|          |             | GO:0005887 | Integral component of plasma membrane | 890  | 499  | 3.16e-06 | 0.02  |
| KEGG     | Pre-diagnosis | path:hsa04360 | Axon guidance | 181  | 114  | 6.91e-05 | 0.02  |
|          | Diagnosis    | path:hsa04360 | Axon guidance | 181  | 133  | 3.67e-06 | 0.001 |
|          |             | path:hsa04713 | Circadian entrainment | 97   | 73   | 1.02e-04 | 0.009 |
|          |             | path:hsa05200 | Pathways in cancer | 531  | 321  | 5.76e-05 | 0.009 |
|          |             | path:hsa05412 | Arrhythmic right ventricular cardiomyopathy | 77   | 60   | 7.83e-05 | 0.009 |
|          |             | path:hsa04020 | Calcium signaling pathway | 193  | 128  | 1.81e-04 | 0.01  |
|          |             | path:hsa04660 | T cell receptor signaling pathway | 104  | 73   | 2.07e-04 | 0.01  |
|          |             | path:hsa04010 | MAPK signaling pathway | 295  | 189  | 2.83e-04 | 0.01  |
|          |             | path:hsa04015 | RAP1 signaling pathway | 210  | 141  | 4.06e-04 | 0.02  |
|          |             | path:hsa04921 | Oxytocin signaling pathway | 153  | 106  | 3.98e-04 | 0.02  |
|          |             | path:hsa04024 | cAMP signaling pathway | 216  | 137  | 9.35e-04 | 0.03  |
|          |             | path:hsa04014 | Ras signaling pathway | 232  | 147  | 1.15e-03 | 0.04  |
|          |             | path:hsa04512 | ECM-receptor interaction | 88   | 63   | 1.62e-03 | 0.045 |
|          |             | path:hsa04072 | Phospholipase D signaling pathway | 148  | 101  | 2.06e-03 | 0.046 |
|          |             | path:hsa04611 | Platelet activation | 124  | 84   | 1.87e-03 | 0.046 |
|          |             | path:hsa04724 | Glutamatergic synapse | 114  | 81   | 1.95e-03 | 0.046 |

Probes ≤0.01 after limma model adjustment for age, sex, and batch effects, which included 15,111 in the pre-diagnosis analysis and 28,787 in the diagnosis analysis.

Abbreviations: cAMP, cyclic adenosine monophosphate; ECM, extracellular matrix; FDR, false discovery rate; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase; Rap1, Ras-proximate-1.

Studies only focus on dementia cases versus controls. However, while this is a novel study, with an average of nearly 3 years between “cognitively healthy” baseline and dementia diagnosed at follow-up, it is not enough time to detect the very early appearance of dementia related neuropathophysiology, thought to begin decades in advance of symptoms. Because of this, future studies should aim to include younger participants in their mid-adulthood, and follow them longitudinally with initial and regular follow-up samples collected, which could be used to measure DNA methylation. Given most cases of dementia occur later in life (after 70 years), such studies will need to be very long and with regular tracking of cognitive function and dementia assessments.

In comparison to some other DNAm studies of dementia, our groups of cases and controls are relatively large. However, because of the large number of methylation probes measured, and thus the need to correct for multiple testing, the study is underpowered to detect the anticipated small effect sizes (< 10%). While raw, unadjusted results were also reported here, the potential for type 1 errors must be considered, as some of these are likely to be chance findings.

Another limitation to consider was the inability to distinguish cases according to the underlying cause of dementia. Future studies should involve large groups of participants with defined causes of dementia, so disease-specific epigenetic profiles can be generated. In older individuals AD is a common cause of dementia, but it is increasingly recognized that many individuals over the age of 80 years likely have mixed dementia, with a complex combination and manifestation of a spectrum of brain conditions, as well as AD pathology. Furthermore, the focus on a white population means the results can not necessarily be generalized to other ethnic groups. Finally, blood is composed of multiple cell types which have different DNAm signatures. No adjustment was made for cell proportions in the analysis because adjusting for cell type removes important biological signatures that may be closely linked to disease etiology.
of the disease itself, and an easy-to-obtain biomarker should be present regardless of a cell type estimation.

There still remains a large gap between DNA methylation biomarker studies and translation to the clinical setting. Replication and validation of these results are needed to refine dementia-specific DNA methylation signatures. In doing so, a polyepigenetic risk score, of high sensitivity and specificity, could be created for both dementia in general, as well as specific diseases which cause dementia.

5 | CONCLUSION

We have identified a distinct DNAm signature which differentiates individuals with and without dementia. Methylation of some sites, gene regions, and genetic pathways may have functional implications in the pathophysiology of dementia. Furthermore, we report that some differentially methylated probes can be detected in blood samples on average 3 years prior to the presence of clinical symptoms, which differentiate those with preclinical dementia compared to controls. More studies are needed to ascertain the true clinical utility of these DNAm signatures and the potential that they could be used in routine screening as an early biomarker of dementia. Future work is now also needed to determine whether these signatures are present even earlier in the disease process (eg, more than 3 years prior to symptom onset).

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CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Anne Murray, Robyn L. Woods, Raj Shah designed and conceptualized the ASPREE study and played a major role in the acquisition of data. Emily Parker and James Phung were responsible for the collection, storage, and retrieval of biological specimens. Joanne Ryan designed this study. Joanne Ryan and Paul Lacaze provided funding. Peter D. Fransquet undertook all EWAS data cleaning and analysis and wrote the manuscript, with assistance from Joanne Ryan. All authors provided intellectual input into the final manuscript.

REFERENCES

1. Duong S, Patel T, Chang F. Dementia: what pharmacists need to know. Can Pharm J (Ott). 2017;150(2):118-129.
2. Ryan J, Fransquet P, Wrigglesworth J, Lacaze P. Phenotypic heterogeneity in dementia: a challenge for epidemiology and biomarker studies. Front Public Health. 2018;6(181).
3. Robinson L, Tang E, Taylor J-P. Dementia: timely diagnosis and early intervention. BMJ (Clinical research ed). 2015;350:h3029-h3029.
4. Perl DP. Neuropathology of Alzheimer’s disease. Mt Sinai J Med. 2010;77(1):32-42.
5. Zetterberg H, Burnham SC. Blood-based molecular biomarkers for Alzheimer’s disease. Molecular Brain. 2019;12(1):26.
6. Watson R, Bryant J, Sanson-Fisher R, Mansfield E, Evans T-J. What is a ‘timely’ diagnosis? Exploring the preferences of Australian health service consumers regarding when a diagnosis of dementia should be disclosed. BMC Health Serv. Res. 2018;18(1):612.
7. Huang L-K, Chao C-H, Tollefsbol TO. Epigenetic biomarkers: current strategies and future challenges for their use in the clinical laboratory. Crit Rev Clin Lab Sci. 2017;54(7-8):529-550.
8. García-Gimenez JL, Seco-Cervera M, Tollefsbol TO, et al. Epigenetic biomarkers: current strategies and future challenges for their use in the clinical laboratory. Crit Rev Clin Lab Sci. 2017;54(7-8):529-550.
9. Moore LD, Le T, Fan G. DNA methylation and its basic function. Neuropsychopharmacology. 2013;38(1):23-38.
10. Li P, Marshall L, Oh G, et al. Epigenetic dysregulation of enhancers in neurons is associated with Alzheimer’s disease pathology and cognitive symptoms. Nat Commun. 2019;10(1):2246.
11. Fransquet PD, Ryan J. Micro RNA as a potential blood-based epigenetic biomarker for Alzheimer’s disease. Clin Biochem. 2018;58:5-14.
12. Fransquet PD, Ryan J. The current status of blood epigenetic biomarkers for dementia. Crit Rev Clin Lab Sci. 2019;56(7):435-457.
13. Fransquet PD, Lacaze P, Saffery R, McNeil J, Woods R, Ryan J. Blood DNA methylation as a potential biomarker of dementia: a systematic review. Alzheimers Dement. 2018;14(1):81-103.
14. McNeil JJ, Woods RL, Nelson MR, et al. Baseline characteristics of participants in the ASPREE (ASPrin in reducing events in the elderly) study. J Gerontol A Biol Sci Med Sci. 2017;72(11):1586-1593.
15. Teng EL, Chui HC. The modified mini-mental state (3MS) examination. J Clin Psychiatry. 1987;48(8):314-318.
16. Ryan J, Woods RL, Britt C, et al. Normative performance of healthy older individuals on the modified mini-mental state (3MS) examination according to ethno-racial group, gender, age, and education level. Clin Neuropsychol. 2019;33(4):779-797.
17. Smith A. Symbol Digit Modalities Test: Manual. Los Angeles: Western Psychological Services; 1982.
18. Ruff RM, Light RH, Parker SB, Levin HS. Benton controlled oral word association test: reliability and updated norms. Arch Clin Neuropsychol. 1996;11(4):329-338.

19. Benedict RHB, Schretlen D, Groninger L, Brandt J. Hopkins. Verbal learning test - revised: normative data and analysis of inter-Form and test-retest reliability. Clin Neuropsychol. 1998;12(1):43-55.

20. Ryan J, Woods RL, Murray AM, et al. Normative performance of older individuals on the Hopkins verbal learning test-revised (HVLT-R) according to ethno-racial group, gender, age and education level. Clin Neuropsychol. 2020;1-17.

21. First MB, Frances A, Pincus HA. DSM-IV-TR Handbook of Differential Diagnosis. Arlington, VA: American Psychiatric Publishing, Inc; 2002.

22. Maksmovic J, Phipson B, Oshlack A. A cross-package bioconductor workflow for analysing methylation array data. F1000Research. 2016;5:1281.

23. Aryee MJ, Jaffe AE, Corrada-Bravo H, et al. Minfi: a flexible and comprehensive bioconductor package for the analysis of infinium DNA methylation microarrays. Bioinformatics. 2014;30(10):1363-1369.

24. Touleimat N, Tost J. Complete pipeline for illumina(R) human methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation. Epigenomics. 2012;4(3):325-341.

25. Pidsley R, Zotenko E, Peters TJ, et al. Critical evaluation of the illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. Genome Biol. 2016;17(1):208.

26. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9(1):559.

27. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.

28. Saffery R, Gordon L. Time for a standardized system of reporting sites of genomic methylation. Genome Biol. 2015;16(1):85.

29. Peters TJ, Buckley MJ, Statham AL, et al. De novo identification of differentially methylated regions in the human genome. Epigenomics. 2015;8:6.

30. Stouffer SA, Suchman EA, Devinney LC, Star SA, Williams Jr RM. The American Soldier: Adjustment During Army Life. (Studies in Social Psychology in World War II) American Soldier: Adjustment During Army Life. (Studies in Social Psychology in World War II) Oxford: Princeton University Press; 1949.

31. Phipson B, Maksmovic J, Oshlack A. missMethyl: an R package for analyzing data from illumina’s HumanMethylation450 platform. Bioinformatics. 2016;32(2):286-288.

32. Lang L, Clifford A, Wei L, et al. Prevalence and determinants of undetected dementia in the community: a systematic literature review and a meta-analysis. BMJ open. 2017;7(2):e011146-e011146.

33. Wang J, Zhao S, He W, et al. A transcription factor IIa-binding site differentially regulates RNA polymerase II-mediated transcription in a promoter context-dependent manner. J Biol Chem. 2017;292(28):11873-11885.

34. Gogoi P, Mordina P, Kanaujia SP. Structural insights into the catalytic mechanism of 5-methylthioribose 1-phosphate isomerase. J Struct Biol. 2019;205(1):67-77.

35. Masliah E, Dumaop W, Galasko D, Desplats P. Distinctive patterns of DNA methylation associated with Parkinson disease: identification of concordant epigenetic changes in brain and peripheral blood leukocytes. Epigenetics. 2013;8(10):1030-1038.

36. Chen GF, Xu TH, Yan Y, et al. Amyloid beta: structure, biology and structure-based therapeutic development. Acta Pharmacol Sin. 2017;38(9):1205-1235.

37. Wahler A, Beyer AS, Keller IE, et al. Engagement adaptor phosphotyrosine-binding-domain-containing 1 (GULP1) is a nucleocytoplasmic shuttling protein and is transactivationally active together with low-density lipoprotein receptor-related protein 1 (LRP1). Biochem J. 2013;450(2):333-343.

38. Kanekisa Laboratories. hsa04360 Pathway: Axon guidance – Homo sapiens (human). 2019; https://www.genome.jp/dbget-bin/www_bget?pathway=hsa04360. Accessed 29 November 2019.

39. Wei YL, Sun H. Identification of hsa-mir-34a, hsa-mir-124, and hsa-mir-204 as signatures for cataract. J Cell Physiol. 2019;234(7):10709-10717.

40. Maharani A, Dawes P, Nazroz J, Tampubolon G, Pendleton N. Cataract surgery and age-related cognitive decline: a 13-year follow-up of the English longitudinal study of ageing. PLoS One. 2018;13(10):e0204833.

41. Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annu Rev Pharmacol Toxicol. 2010;50:295-322.

42. Brooks LKR, Mias GI. Data-Driven analysis of age, sex, and tissue effects on gene expression variability in Alzheimer’s disease. Front. Neurosci. 2019;13:392.

43. Archetti D, Ingala S, Venkatraghavan V, et al. Multi-study validation of data-driven disease progression models to characterize evolution of biomarkers in Alzheimer’s disease. Neurolmage: Clinical. 2019;24:101954.

44. Hou Y, Dan X, Babbar M, et al. Ageing as a risk factor for neurodegenerative disease. Nat Rev Neurol. 2019;15(10):565-581.

45. Kapasi A, Schneider JA. Vascular contributions to cognitive impairment, clinical Alzheimer’s disease, and dementia in older persons. Biochim. Biophys. Acta, Mol. Basis Dis. 2016;1862(5):878-886.

46. Attems J, Jellinger KA. The overlap between vascular disease and Alzheimer’s disease–lessons from pathology. BMC Med. 2014;12:206-206.

47. Galanter JM, Gignoux CR, Oh SS, et al. Differential methylation between ethnic sub-groups reflects the effect of genetic ancestry and environmental exposures. Elife. 2017;6:e20532.

48. Holbrook JD, Huang RC, Barton SJ, Saffery R, Lillycrop KA. Is cellular heterogeneity merely a confounder to be removed from epigenome-wide association studies. Epigenomics. 2017;9(8):1143-1150.

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