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DNA Methylation in the Apolipoprotein-A1 Gene is Associated with Episodic Memory Performance in Healthy Older Individuals

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Abstract.
Background: DNA methylation variation has been implicated in memory, cognitive performance, and dementia. Plasma apolipoprotein-A1 (ApoA1) levels may act as a biomarker of age-associated cognitive performance and decline.
Objectives: To estimate the heritability of plasma ApoA1 protein levels; to examine DNA methylation variation within the *APOA1* gene; and to investigate whether *APOA1* methylation is associated with plasma ApoA1 levels and episodic memory performance.
Method: Heritability of ApoA1 protein levels in Older Australian Twins Study (OATS) was assessed using structural equation modelling. *APOA1* methylation levels were assayed in two cohorts of cognitively normal older individuals. The methylation status of 12 CpGs in 24 twin pairs from OATS was assayed using the Illumina 450K methylation array. Candidate CpGs were assayed in 454 individuals from Sydney Memory and Ageing Study (Sydney MAS) using pyrosequencing. Regression analyses assessed associations between *APOA1* methylation levels, ApoA1 plasma levels, and memory performance.
Results: No significant heritability was observed for ApoA1 protein levels. *APOA1* candidate-gene analyses revealed CpG sites associated with memory performance in the twin study ($p < 0.050$). Replication of an association between methylation of a specific CpG (cg03010018) in *APOA1* and memory performance was observed in Sydney MAS ($\beta = -0.145$, $p = 0.010$).

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Methylation of this CpG site was also significantly correlated with ApoA1 protein levels ($\beta = 0.161$, $p = 0.019$). However, no relationship between a composite memory domain score and methylation was observed ($p = 0.389$).
Conclusion: Findings demonstrated that epigenetic control of *APOA1* expression and DNA methylation levels are associated with episodic memory performance in older adults.

Keywords: Aging, apolipoprotein A1, DNA methylation, epigenomics, episodic memory

INTRODUCTION

Aging is associated with increased cognitive decline [1], which may be an early marker of dementia, such as Alzheimer's disease (AD) [2]. Despite animal studies that have implicated epigenetic mechanisms, such as DNA methylation, in age-related cognitive decline [3, 4], only three human studies have examined leukocyte DNA methylation and cognitive performance in healthy adults. Schiepers and colleagues [5] found no significant association between global DNA methylation and any of several key domains of cognitive function in healthy older adults. Another study reported no significant association between performance on the Mini-Mental State Examination (MMSE) and global methylation in older adults [6]. Finally, Yu and colleagues [7] investigated whether or not DNA methylation is associated with human intelligence, in a Japanese cohort of young monozygotic twins. One differentially methylated gene was found: Rho GTPase activating protein 18 (*ARHGAP18*) gene. However, the study did not test other important cognitive domains such as memory, and only examined promoter regions in 59% of CpG islands in the genome. Moreover, age-related cognitive performance was not examined.

Apolipoproteins are multi-functional glycoproteins that bind lipids and are associated with various functions, especially lipid metabolism [8]. Major protein classes include apolipoproteins A, B, C, E, H, and J (clusterin). Evidence from animal and human studies suggests apolipoproteins are involved in age-related cognitive performance and decline. The APOE $\varepsilon 4$ polymorphism is widely accepted as a genetic risk factor for age-related cognitive performance and decline [9]. However, additional apolipoproteins have recently been implicated in cognitive decline. Song and colleagues [10] examined the levels of plasma apolipoproteins A1, A2, B, C3, E, H, and J, and cognition in a longitudinal community-based cohort with a mean age of 78 years. Results demonstrated that multiple apolipoprotein levels predicted cognitive performance and decline. Specifically, low plasma *ApoA1* levels were significantly associated with mild cognitive impairment (MCI) and predicted conversion to Clinical Dementia Rating scale >0 over a 2 year period [10]. A related study using the proteomics iTRAQ platform confirm that plasma ApoA1 levels were dysregulated in amnestic MCI and AD, suggesting a role for ApoA1 in memory aspects of cognition [11]. Consistent with the role of ApoA1 in AD, Lefterov and colleagues [12] demonstrated that deletion of mouse *APOA1* exacerbates memory deficits, and secondly that ApoA1 is protective against amyloid-β toxicity in brain vascular smooth muscle cells.

In this study, we utilized the advantages of a discordant-phenotype monozygotic (MZ) twin study to examine the relationship between DNA methylation and memory performance. MZ twin studies represent a unique resource for epigenetics research and are considerably more powerful than population-based singleton studies [13], as the effects of important epigenetic confounders such as age, sex, early environmental experiences, and genetic background, are significantly reduced [14]. Hence, the discordant MZ twin design is a powerful tool to decipher the role of epigenetics and/or environment on a particular phenotype such as memory performance in humans. The current study is an extension of work conducted by Song and colleagues [10] to examine whether DNA methylation of the *APOA1* locus is associated with ApoA1 protein levels and memory performance in older adults. Here we examined whether ApoA1 protein levels are heritable in the Older Australian Twins Study (OATS). We then examined whether differentially methylated regions in the *APOA1* gene were associated with memory performance using the discordant MZ twin design. Replication of the *APOA1* CpG results was then undertaken in an independent cohort of cognitively healthy, community-based older individuals, the Sydney Memory and Aging Study (Sydney MAS). We also assessed whether *APOA1* DNA methylation variation influenced ApoA1 plasma protein levels.

METHODS AND MATERIALS

Older Australian Twins Study

The OATS is described in detail in a previous publication [15]. Participants who were aged 65 and over

were recruited into OATS through the Australian Twin Registry and a recruitment drive from the three eastern states of Australia (Victoria, New South Wales, Queensland). Phenotypic data including demographics, medical history, and performance on a battery of cognitive assessments were collected. Peripheral blood samples were donated for DNA extraction and blood biochemistry. DNA was extracted using standard methods by Genetic Repositories Australia. Written informed consent was provided by all participants and ethics approval was given by the relevant ethics committees (Australian Twin Registry, University of New South Wales, University of Melbourne, QIMR Berghofer Medical Research Institute, and the South Eastern Sydney and Illawarra Area Health Service). Inclusion criteria included a co-twin who consented to participate and had completed some education in English. Exclusion criteria included a current diagnosis of an acute psychosis or insufficient English to complete the cognitive assessments. Further details of the study are provided in Sachdev et al. [15].

Sydney memory and ageing study (sydney MAS)

Participants in this study were drawn from the Sydney MAS, which comprised community-based participants aged 70–90 years, who were randomly recruited from the electoral roll in two regions of Sydney, Australia. Data include results from demographic information and validated neuropsychological tests. Peripheral blood samples were collected for DNA extraction and blood biochemistry analysis. DNA was extracted using standard methods by Genetic Repositories Australia. Written informed consent was provided by all participants and ethics approval was issued by the relevant ethics committees (University of New South Wales, South Eastern Sydney and Illawarra Area Health Service). For further details see Sachdev et al. [16].

ApoA1 protein levels

Serum ApoA1 protein levels in both cohorts were measured using an immunoassay method as described in Song et al. [11]. Current use of lipid-lowering drugs (hypolipidemic medications) was also determined via self-report, which included the following medications: Atorvastatin Calcium, Cholestyramine, Ezetimibe, Fenofibrate, Fluvastatin Sodium, Gemfibrozil, Pravastatin Sodium, Rosuvastatin Calcium, and Simvastatin.

Heritability of ApoA1 protein levels

Heritability is defined as the ratio of additive genetic variance to the total phenotypic variance. Twin data enables us to model the phenotypic covariance between the twin pairs as a function of additive genetic (A), shared environmental (C), and unique environmental (E) components as the MZ and dizygotic (DZ) twins, respectively, are known to share 100% and 50% of their genetic material. The three parameters (A, C, and E) can be estimated using Structural equation models (SEM), known as the ACE model [17]. For parsimony, models containing the variance components A and E (AE), C and E (CE), and E are usually compared with the full ACE model. Here heritability for plasma ApoA1 levels was estimated using 68 MZ and 46 DZ pairs (63 female, 32 male same-sex pairs and 19 opposite sex pairs) from OATS. The package OpenMx was used to fit the ACE model for heritability using the covariates age, sex, batch number of assay, and hypolipidemic medication.

Cognitive measures

Two cognitive measures were examined in this study, an episodic memory test, Logical Memory Story A delayed recall (LMDR) [18] and a composite memory domain measure. The LMDR is routinely used as a test of episodic memory [19] in both cohorts. A composite memory domain score for Sydney MAS was calculated from the average of the Z-scores of following tests: LMDR, Rey Auditory Verbal Learning Test (RAVLT) [20] total learning (trials 1–5), RAVLT short-term delayed recall (trial 6), RAVLT long-term delayed recall (trial 7), and the Benton Visual Retention Test (recognition) [21].

Memory-discordant MZ twin pairs

OATS discordant MZ twin pairs ($n = 24$) for episodic memory were defined as co-twins whose absolute difference for the LMDR [18] scores was ≥ 1 SD. This test was chosen since: (i) prior evidence suggests that epigenetics is implicated in memory and learning [22] and; (ii) this cognitive test showed the highest discordance between twin pairs within our OATS sample.

Determination of APOA1 CpG methylation by hybridization array

Methylation levels of the 12 *APOA1* CpGs were determined from peripheral blood DNA samples from

24 pairs of OATS LMDR-discordant twins. Individual data for each CpG were extracted from whole-genome data generated by Illumina 450K methylation array using an established genomics provider. DNA methylation was estimated as M-values, defined as the log ratio of the methylated over unmethylated channels [23].

Pyrosequencing

Replication of the results observed for the memory-discordant MZ twin study was undertaken in Sydney MAS ($n = 454$). DNA methylation was assessed by the CpG site-specific pyrosequencing technique. Primers were designed to encompass the identified CpG sites using the PyroMark® Assay Design Software 2.0 (QIAGEN, Hilden, Germany). The primers designed for PCR amplification and pyrosequencing, including forward (F), reverse (R), and sequencing (SEQ) primers are shown in Supplementary Table 1. MAS DNA samples were bisulphite-converted in preparation for pyrosequencing using the Epitect® 96 Bisulphite Kit (QIAGEN). Target genomic regions of bisulphite-treated DNA were amplified using specific primers. Touchdown Polymerase Chain Reaction (T-PCR) was used to amplify the regions of interest using the PyroMark® PCR Kit (QIAGEN) in accordance with the manufacturer's protocol. Agarose gel electrophoresis confirmed the expected size of the amplified PCR product.

Pyrosequencing of the PCR products was then performed using the appropriate sequencing primers to estimate DNA methylation variation of the target CpG sites. Pyrosequencing was carried out using the QIAGEN PyroMark® Q24 Pyrosequencing System following the manufacturer's protocol using 5 μl of PCR product. Accuracy and reliability of the pyrosequencing technique was confirmed by intra-assay triplicate experiments using eight control DNA samples run on a single plate.

Statistical analyses

The analysis of candidate *APOA1* CpG sites available on the 450K array chip in the memory-discordant twins was conducted in R using the *minfi* and *limma* packages. Analyses were undertaken to assess the relationships between methylation (M-values) of the *APOA1* CpG sites and memory performance using a paired analysis in the *limma* [24]. All other analyses were undertaken using IBM® SPSS® Statistics Version 21. Correlation, and its (two-tailed) significance, was assessed using Pearson's correlation coefficient. Multivariate linear regression analyses were undertaken in Sydney MAS to assess the relationships between DNA methylation levels and (i) ApoA1 protein levels and (ii) memory test performance. Potential confounders were age, sex, years of education, non-English speaking background (NESB) status, hypolipidemic medication, and plate to plate variability (batch effects). ApoA1 plasma levels were significantly correlated with sex, with higher levels observed for females than males. Batch effects were evident for *APOA1* methylation. Age, sex, years of education, and NESB status were correlated with LMDR and memory domain scores. Hypolipodemic medication was only correlated with sex. Therefore, the final covariates used in multiple regression models were those which were significantly correlated with either the dependent or predictor variables, as well as the *a priori* covariates of age and sex. For all statistical analyses, significance was defined as $p \leq 0.05$.

RESULTS

Heritability of plasma ApoA1 levels

Descriptive statistics of the OATS sample ($n = 136$ MZ and $n = 92$ DZ twins) are shown in Table 1. Heritability analysis for plasma ApoA1 levels shows environmental influence on this trait is higher compared to the additive genetic and shared environmental influences. Comparison of the likelihoods of the full ACE model versus the reduced models AE ($p = 1.000$), CE ($p = 0.720$), and E ($p = 0.840$) shows that the model with the unique environmental component adequately explains the trait variability. Considering the model parsimony based on the maximum p-value, the heritability estimate under the AE model is found to be 0.07 (95% CI is 0–0.295). The covariates, sex ($p = 0.030$) and batch ($p < 0.001$), were found to be significant and all other covariates were not ($p = 0.600$–0.950).

Identification of candidate APOA1 CpGs using memory-discordant MZ twin pairs

Twenty-four MZ pairs discordant for episodic memory performance (*Mean LMDR score* = 11.02, SD = 5.32, *range* = 1–23), aged 65–79 years (*Mean* = 68.48, *SD* = 3.45) and comprising 18 male and 30 females, were examined in these analyses. Of the 12 *APOA1* associated probes (Illumina 450K manifest), methylation levels of two *APOA1* CpG sites were significantly correlated with LMDR scores (cg03010018 (hg19, chr11:116,708,300) located in the

Table 1
Demographic data for OATS and Sydney MAS cohorts

	OATS		MAS
	Monozygotic	Dizygotic	
N	136	92	454
Male *n* (%)	58 (42.6)	25 (27.2)	226 (50%)
Age,	70.95 ± 5.67	69.83 ± 4.50	78.34 ± 4.65
Mean ± *SD* (range)	(65–88)	(65–81)	(70–90)
Years of education,	10.77 ± 2.83	10.73 ± 3.02	11.86 ± 3.61
Mean ± *SD* (range)	(6–19)	(5–20)	(4–24)
Non-English-speaking background, *n* (%)	0	0	15%
APOA1-[CpG1] methylation %,	N/A[b]	N/A[b]	78.08 ± 8.36
Mean ± *SD* (range)			(59–100)
ApoA1 plasma levels (μg/mL), *Mean* ± *SD*	2857.07 ± 2466.59	2414.44 ± 1683.95	2745.23 ± 1173.58
LMDR scores,	9.63 ± 3.89	9.90 ± 3.73	9.43 ± 3.98
Mean ± *SD* (range)	(1–21)	(1–16)	(0–20)
Memory Domain score[a],	N/A	N/A	–0.51 ± 1.18
Mean ± *SD* (range)			(–4.12–2.43)
Currently taking hypolipidemic medication	52 (44.4)	26 (30.2)	245 (54.1%)

N/A, not applicable; [a]z-score; [b]values were omitted as data from Illumina chip were not directly comparable with pyrosequencing analysis.

5'UTR (log fold change M value = –0.135; $p = 0.012$; cg20200605 (hg19, chr11:116,706,865) located in the gene body, log fold change M value = 0.157; $p = 0.013$). Pair-wise comparisons of the M values of the 12 CpGs in the 48 individuals revealed that the CpGs were not independent but multiple CpGs displayed significant correlation in their methylation levels (Pearson correlation coefficient $r > |0.3|$; $p < 0.050$) as shown in Supplementary Table 2. Of note, two CpGs that were significantly associated with LMDR (cg03010018 and cg20200605) had correlated methylation values ($r = –0.41$; $p = 0.005$). The relationships between ApoA1 protein levels and the other two parameters, *APOA1* methylation (linear regression coefficient = +161.558; $p > 0.050$), and LMDR score (linear regression coefficient = –5.271 e-04; $p > 0.050$), were not significant. However, care should be taken in the interpretation of the results, as protein data was not available for 27% (13/48) of the OATS discovery cohort. The two CpG sites (cg03010018 and cg20200605) were selected for replication in the Sydney MAS cohort using pyrosequencing.

Examination of APOA1 methylation levels by pyrosequencing in Sydney MAS cohort

After optimization of both PCR and pyrosequencing reactions by systematic variations in buffer and annealing conditions, only one CpG site (cg03010018) was suitable for further investigation as the second site (cgcg20200605) failed quality control. The average intra-assay coefficient of variation for pyrosequencing of this *APOA1* CpG site was 2.85%, indicating high reliability and reproducibility of the pyrosequencing. The observed mean *APOA1* methylation value for the entire sample was 78.08% (SD = 8.36) ranging from 59–100%. Descriptive statistics for the Sydney MAS sample with methylation data are presented in Table 1.

Using multiple linear regression, a significant association between *APOA1*-methylation and ApoA1 protein levels was observed ($\beta = 0.157$ $p = 0.022$; covariates age, sex, and batch effects; Fig. 1a). *APOA1* methylation was also a significant predictor of LMDR scores, with an inverse relationship observed ($\beta = –0.132$, $p = 0.019$; covariates age, sex, years of education, batch effects, and NESB status; Fig. 1b). In contrast, no significant relationship was observed between methylation and memory domain scores when analyses were adjusted for the same covariates ($\beta = 0.010$, $p = 0.855$; Fig. 1c).

Of interest, there was no significant relationship between ApoA1 plasma protein levels and: i) LMDR test scores ($\beta = 0.009$, $p = 0.864$); or ii) memory domain scores ($\beta = –0.062$, $p = 0.231$) after adjusting for age, sex, years of education, and NESB status.

DISCUSSION

In this study, the role *APOA1* methylation plays in age-related memory performance was investigated in older adults. Using a discordant MZ twin design, in a candidate gene analysis, we identified two putative CpG sites which were differentially methylated

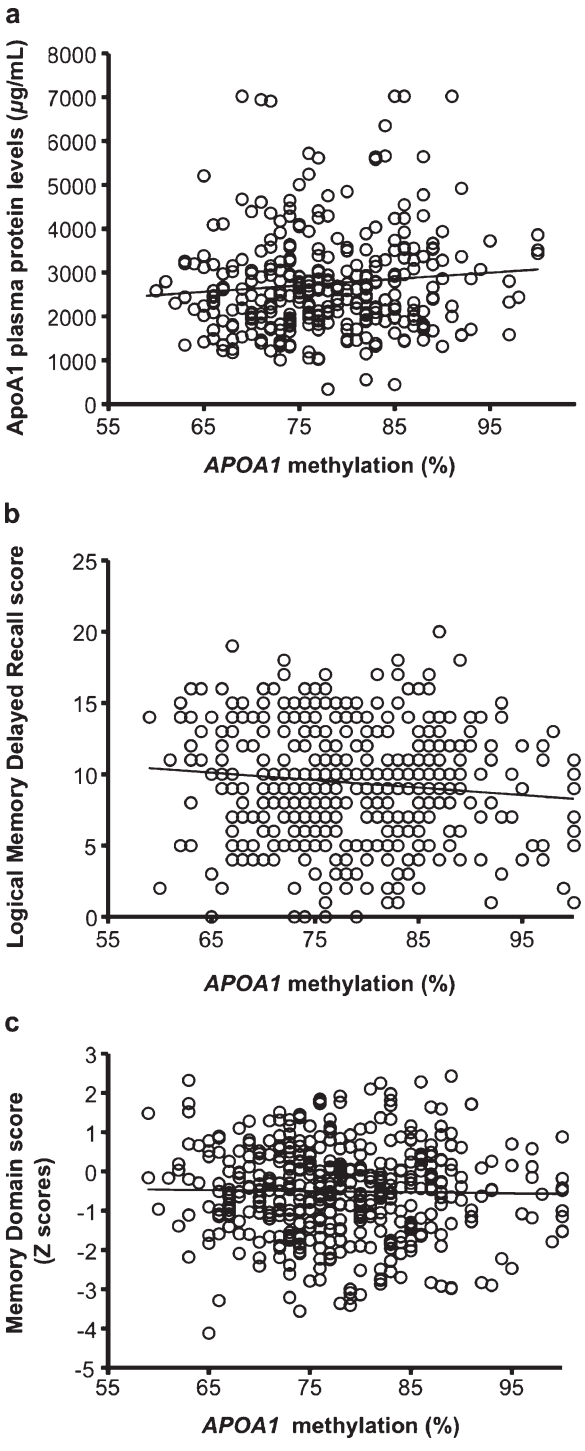

Fig. 1. Scatter plots illustrating relationships (Pearson correlation coefficient (r) and corresponding p value) between (a) plasma Apo-A1 protein levels and *APOA1* DNA methylation ($r = 0.101$, $p = 0.073$); (b) Logical Memory Delayed Recall test scores and *APOA1* DNA methylation ($r = 0.111$, $p = 0.019$); (c) Memory Domain scores and *APOA1* DNA methylation ($r = -0.018$, $p = 0.705$).

and associated with memory performance. One of these CpG sites was pyrosequenced successfully in an independent sample of older adults and the association with memory performance replicated. Interestingly, plasma ApoA1 levels did not appear to be heritable in our cohort of older adults, suggesting factors such as epigenetic variation rather than genetic polymorphisms play a major role in determining ApoA1 levels. Consistent with this finding, differential DNA methylation of the *APOA1* CpG site was significantly associated with ApoA1 protein levels in our study. Epigenetic mechanisms within other lipoprotein genes, such as the *Phospholipid transfer protein* gene, have also been demonstrated to contribute independently to plasma lipid levels [25]. Finally, epigenetic mechanisms that are sensitive to environmental influences and age, may explain the wide variation in estimates of heritability of ApoA1 and other lipoproteins [26].

The identified *APOA1*-CpG site is located in the 5' untranslated region of the gene and hence is transcribed into mRNA but not translated. This site may potentially affect gene expression and consequently, protein levels. Our results are consistent with this premise suggesting differential methylation may play a role in influencing gene expression. However, contrary to expectations, a positive correlation was observed with higher levels of methylation linked to increased ApoA1 levels. A possible explanation is that DNA methylation at this particular CpG site may be involved in the "switching-on" of the target gene, resulting in increased gene expression and synthesis of the encoded protein. Support for this phenomenon can be found in recent literature [27] that suggests both positive and negative correlations can be observed between DNA methylation and gene expression in CpG sites located near the transcription site and in the gene body [27]. The exact mechanisms for this process are yet to be understood and warrant further investigation. A limitation was that we examined the relationship between *APOA1* methylation and ApoA1 protein levels, and not *APOA1* transcript levels. Post-translational modifications [28] may alter the protein independently of transcript levels and obscure the relationship between *APOA1* methylation and gene expression.

A significant inverse association between DNA methylation of *APOA1*-CpG and episodic memory (LMDR performance) was observed in two independent cohorts of older adults. However, there was no significant relationship between a composite memory domain measure, comprising scores from multiple memory tests, and *APOA1*-CpG DNA methylation. Since the memory domain measure included additional

tests of memory performance, such as visual and short-term memory tests, the finding in this study and the preliminary data from OATS may only be relevant to verbal delayed recall as represented by the LMDR.

The direction of our results is to some extent inconsistent with earlier results from Song et al. [11] that found lower plasma ApoA1 protein levels were associated with MCI and predicted cognitive decline. We found that poorer episodic memory was associated with increased DNA methylation and hence higher levels of ApoA1 protein although ApoA1 protein levels were not significantly associated with this specific memory performance test. In spite of this observation, recent research demonstrated ApoA1 protein levels are associated with MCI and increased risk of cognitive decline over 2 years [11]. A possible explanation for this unexpected finding is that memory performance may not only be associated with solely with *APOA1*-CpG methylation, but the consequences of variation in DNA methylation patterns of multiple loci. Another possibility is that key regulators of global methylation levels, such as the DNA methyltransferases, may be involved. Dysregulation of *DNMT3A* expression has previously been associated with neurodegenerative diseases such as frontotemporal dementias [29]. Moreover, using mice models, Oliveira and colleagues [22] demonstrated that DNA-methyltransferase 3a2 (DNMT3a2), an important regulator of DNA methylation patterns in the genome, is associated with age-related cognitive decline. By increasing expression of DNMT3a2 the authors were able to restore cognitive performance in aged mice, suggesting an association between DNA methylation patterns and age-related cognitive performance in mice.

Limitations of the current study include the use of leukocyte-derived DNA for examining memory performance, a brain-related phenotype. However, there are obvious difficulties in sampling brain tissue from living volunteers and there is some evidence suggesting blood DNA samples may be utilized as a proxy for other tissues [30]. From the current study it is not possible to ascertain whether DNA methylation changes to the *APOA1* gene are causally related to memory performance, or whether it is an epiphenomenon.

Although we have shown that *APOA1* DNA methylation is associated with age-related verbal episodic memory performance, the mechanism driving this relationship remains unclear. Future studies should examine longitudinal DNA methylation changes and whether or not these changes are linked to decline in age-related memory performance. Ultimately, the identification of epigenetic marks that may predispose individuals to age-related cognitive decline and dementia may distinguish at risk individuals and facilitate preventative and treatment interventions in the future.

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SUPPLEMENTARY MATERIAL

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REFERENCES

[1] Morrison JH, Baxter MG (2012) The ageing cortical synapse: Hallmarks and implications for cognitive decline. *Nat Rev Neurosci* **13**, 240-250.

[2] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH

(2011) The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263-269.

[3] Penner MR, Roth TL, Barnes CA, Sweatt JD (2010) An epigenetic hypothesis of aging-related cognitive dysfunction. *Front Aging Neurosci* **2**, 9.

[4] Lubin FD (2011) Epigenetic gene regulation in the adult mammalian brain: Multiple roles in memory formation. *Neurobiol Learn Mem* **96**, 68-78.

[5] Schiepers OJ, van Boxtel MP, de Groot RH, Jolles J, Kok FJ, Verhoef P, Durga J (2012) DNA methylation and cognitive functioning in healthy older adults. *Br J Nutr* **107**, 744-748.

[6] Gomes MV, Toffoli LV, Arruda DW, Soldera LM, Pelosi GG, Neves-Souza RD, Freitas ER, Castro DT, Marquez AS (2012) Age-related changes in the global DNA methylation profile of leukocytes are linked to nutrition but are not associated with the MTHFR C677T genotype or to functional capacities. *PLoS One* **7**, e52570.

[7] Yu CC, Furukawa M, Kobayashi K, Shikishima C, Cha PC, Sese J, Sugawara H, Iwamoto K, Kato T, Ando J, Toda T (2012) Genome-wide DNA methylation and gene expression analyses of monozygotic twins discordant for intelligence levels. *PLoS One* **7**, e47081.

[8] Davidsson P, Hulthe J, Fagerberg B, Camejo G (2010) Proteomics of apolipoproteins and associated proteins from plasma high-density lipoproteins. *Arterioscler Thromb Vasc Biol* **30**, 156-163.

[9] Wisdom NM, Callahan JL, Hawkins KA (2011) The effects of apolipoprotein E on non-impaired cognitive functioning: A meta-analysis. *Neurobiol Aging* **32**, 63-74.

[10] Song F, Poljak A, Kochan NA, Raftery M, Brodaty H, Smythe GA, Sachdev PS (2014) Plasma protein profiling of Mild Cognitive Impairment and Alzheimer's disease using iTRAQ quantitative proteomics. *Proteome Sci* **12**, 5.

[11] Song F, Poljak A, Crawford J, Kochan NA, Wen W, Cameron B, Lux O, Brodaty H, Mather K, Smythe GA, Sachdev PS (2012) Plasma apolipoprotein levels are associated with cognitive status and decline in a community cohort of older individuals. *PLoS One* **7**, e34078.

[12] Lefterov I, Fitz NF, Cronican AA, Fogg A, Lefterov P, Kodali R, Wetzel R, Koldamova R (2010) Apolipoprotein A-I deficiency increases cerebral amyloid angiopathy and cognitive deficits in APP/PS1DeltaE9 mice. *J Biol Chem* **285**, 36945-36957.

[13] Bell JT, Spector TD (2011) A twin approach to unraveling epigenetics. *Trends Genet* **27**, 116-125.

[14] Milosavljevic A (2011) Emerging patterns of epigenomic variation. *Trends Genet* **27**, 242-250.

[15] Sachdev PS, Lammel A, Trollor JN, Lee T, Wright MJ, Ames D, Wen W, Martin NG, Brodaty H, Schofield PR, team Or (2009) A comprehensive neuropsychiatric study of elderly twins: The Older Australian Twins Study. *Twin Res Hum Genet* **12**, 573-582.

[16] Sachdev PS, Brodaty H, Reppermund S, Kochan NA, Trollor JN, Draper B, Slavin MJ, Crawford J, Kang K, Broe GA, Mather KA, Lux O, Memory, Ageing Study T (2010) The Sydney Memory and Ageing Study (MAS): Methodology and baseline medical and neuropsychiatric characteristics of an elderly epidemiological non-demented cohort of Australians aged 70-90 years. *Int Psychogeriatr* **22**, 1248-1264.

[17] Rijsdijk FV, Sham PC (2002) Analytic approaches to twin data using structural equation models. *Brief Bioinform* **3**, 119-133.

[18] Weschler D (1997) *Wechsler Adult Intelligence Scale*, 3rd ed, Harcourt Assessment, San Antonio.

[19] Squire LR, Zola SM (1998) Episodic memory, semantic memory, and amnesia. *Hippocampus* **8**, 205-211.

[20] Rey A (1964) *L'Examen Clinique en Psychologie*, Presses Universitaires de France, Paris.

[21] Benton AL, Sivan AB, Spreen O (1966) *Der Benton Test*, 7th ed, Huber, Bern.

[22] Oliveira AM, Hemstedt TJ, Bading H (2012) Rescue of aging-associated decline in Dnmt3a2 expression restores cognitive abilities. *Nat Neurosci* **15**, 1111-1113.

[23] Du P, Zhang X, Huang CC, Jafari N, Kibbe WA, Hou L, Lin SM (2010) Comparison of Beta-value and M-value methods for quantifying methylation levels by microarray analysis. *BMC Bioinformatics* **11**, 587.

[24] Smyth GK (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* **3**, Article3.

[25] Guay SP, Brisson D, Lamarche B, Gaudet D, Bouchard L (2014) Epipolymorphisms within lipoprotein genes contribute independently to plasma lipid levels in familial hypercholesterolemia. *Epigenetics* **9**, 718-729.

[26] Kettunen J, Tukiainen T, Sarin AP, Ortega-Alonso A, Tikkanen E, Lyytikainen LP, Kangas AJ, Soininen P, Wurtz P, Silander K, Dick DM, Rose RJ, Savolainen MJ, Viikari J, Kahonen M, Lehtimaki T, Pietilainen KH, Inouye M, McCarthy MI, Jula A, Eriksson J, Raitakari OT, Salomaa V, Kaprio J, Jarvelin MR, Peltonen L, Perola M, Freimer NB, Ala-Korpela M, Palotie A, Ripatti S (2012) Genome-wide association study identifies multiple loci influencing human serum metabolite levels. *Nat Genet* **44**, 269-276.

[27] Wagner JR, Busche S, Ge B, Kwan T, Pastinen T, Blanchette M (2014) The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. *Genome Biol* **15**, R37.

[28] Karve TM, Cheema AK (2011) Small changes huge impact: The role of protein posttranslational modifications in cellular homeostasis and disease. *J Amino Acids* **2011**, 207691.

[29] Banzhaf-Strathmann J, Claus R, Mucke O, Rentzsch K, van der Zee J, Engelborghs S, De Deyn PP, Cruts M, van Broeckhoven C, Plass C, Edbauer D (2013) Promoter DNA methylation regulates progranulin expression and is altered in FTLD. *Acta Neuropathol Commun* **1**, 16.

[30] Adkins RM, Thomas F, Tylavsky FA, Krushkal J (2011) Parental ages and levels of DNA methylation in the newborn are correlated. *BMC Med Genet* **12**, 47.