PSYCHOGERIATRICS
The Official Journal of the Japanese Psychogeriatric Society
doi:10.1111/psyg.12440 PSYCHOGERIATRICS 2019; 19: 574–583

ORIGINAL ARTICLE

Association of multiple candidate genes with mild cognitive impairment in an elderly Chinese Uygur population in Xinjiang

Ting ZOU,[1] Wei CHEN,[1] Xiaohui ZHOU,[1] Yali DUAN,[1] Xiuru YING,[2] Guili LIU,[2] Meisheng ZHU,[1] Abuliz PARI,[1] Kader ALIMU,[1] Haijun MIAO,[1] Keyim KABINUR,[1] Lei ZHANG,[1] Qinwen WANG[2] and Shiwei DUAN[2]

[1]Department of Geriatrics, The First Affiliated Hospital of Xinjiang Medical University, Urumqi and [2]Ningbo Key Lab of Behavior Neuroscience, Zhejiang Provincial Key Laboratory of Pathophysiology, School of Medicine, Ningbo University, Ningbo, China

Correspondence: Xiaohui Zhou, Department of Geriatrics, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang Province, China. Email: zhouxiaohui858@sina.com. Qinwen Wang and Shiwei Duan, Ningbo Key Lab of Behavior Neuroscience, Zhejiang Provincial Key Laboratory of Pathophysiology, School of Medicine, Ningbo University, Ningbo, Zhejiang Province, China. Email: wangqinwen@nbu.edu.cn; duanshiwei@nbu.edu.cn

Disclosure: Authors have no conflicts of interest to disclose.

Ting Zou and Wei Chen were co-first authors of this work.

Present address: Dr Ting Zou, Department of Geriatrics, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang Province, China. Email: zt.joanna@163.com. Tel: +13999172605

Ningbo University High Technology Research and Development Projects of Xinjiang Province 201517104

National Natural Science Foundation of China 81360064

K. C. Wong Magna Fund in Ningbo University

Received 11 September 2018; revision received 17 January 2019; accepted 30 January 2019.

Key words: APOE *ε4,* DLST, *interaction, MCI, methylation,* OGG1.

Abstract

Background: Mild cognitive impairment (MCI) is a high-risk factor for Alzheimer's disease (AD). In the present study, we investigated the association of genetic polymorphisms of five genes (8-oxoguanine DNA glycosylase 1 (*OGG1*), bridging integrator 1 (*BIN1*), sortilin-related receptor 1 (*SORL1*), presenilin 2 (*PSEN2*) and nerve growth factor (*NGF*)) with MCI risk in a Xinjiang Uygur population. We also tested the relationship between the promoter methylation of genes *OGG1* and dihydrolipoamide S-succinyltransferase (*DLST*) with MCI.

Methods: This study involved 43 MCI patients and 125 controls. Genotyping was done by Sanger sequencing. DNA methylation assays used quantitative methylation-specific polymerase chain reaction.

Results: We found that polymorphisms of five genes and the methylation of *DLST* and *OGG1* genes were not associated with MCI ($P > 0.05$). Further subgroup analysis found that *DLST* hypomethylation was significantly associated with MCI in the carriers of apolipoprotein E (*APOE*) ε4 ($P = 0.042$). In the carriers of non-*APOE* ε4, *DLST* methylation levels were significantly lower in the male control group than in the female control group ($p = 0.04$). Meanwhile, among the non-*APOE* ε4 carriers younger than 75, *OGG1* hypermethylation levels were significantly associated with MCI ($P = 0.049$). *DLST* methylation in female controls was significantly lower than that in male controls ($P = 0.003$). According to gender stratification, there was a significant positive correlation of fasting plasma glucose (FBG) and high-density lipoprotein (HDL) with *OGG1* methylation in the female controls (FBG: $P = 0.024$; HDL: $P = 0.033$). There was a significant inverse correlation between low-density lipoprotein and *DLST* methylation in male MCI ($P = 0.033$). There was a significant positive correlation between HDL and *DLST* methylation levels in the female controls ($P = 0.000$).

Conclusions: This study was the first to discover that *DLST* promoter methylation interacted with *APOE* ε4 and thus affected the pathogenesis of MCI. In addition, *OGG1* promoter methylation interacted with several other factors to increase the risk of MCI.

INTRODUCTION

Mild cognitive impairment (MCI) is a transitional phase between healthy cognitive aging and Alzheimer's disease (AD).[1] MCI is more likely to develop into AD than in the normal population.[2] The MCI population develops dementia at a rate of

10–15% per year, while the overall population develops 1–2% of dementia per year.[3] About 60% of MCI people develop AD within 10 years.[4] However, no effective treatment for dementia has been found. Therefore, how to diagnose and provide early intervention with MCI is receiving more and more attention.

In recent years, with the in-depth study of the pathogenesis of MCI, genetic factors have received extensive attention in the aetiology of MCI. Identification of a MCI-causing gene will undoubtedly bring new ways to prevent and treat this cognitive disorder. Recent studies have found that genetic polymorphisms are associated with MCI,[5–8] while many previous studies have shown that bridging integrator 1 (*BIN1*), sortilin-related receptor 1 (*SORL1*), presenilin 2 (*PSEN2*) and nerve growth factor (*NGF*) and 8-oxoguanine DNA glycosylase 1 (*OGG1*) gene polymorphisms and protein phenotypes are associated with cognitive decline and nervous system degenerative disease. *BIN1* affects cognitive function by regulating tau protein,[9] which regulates the transport and recycling of amyloid-β (Aβ) protein precursor protein in the pathogenesis of AD and MCI,[10] and its polymorphism is associated with decreased Aβ concentration in cerebrospinal fluid.[11–13] Mutations in the *PSEN2* gene have been identified in association with early-onset AD (EOAD), and recent studies have found that it can also lead to Ca^{2+} homeostasis, which in turn induces neurodegenerative diseases.[14] NGF plays an important role in the pathogenesis of AD.[15] In recent years, studies have found that treatment of AD with NGF can improve cognitive function and decrease the level of Aβ protein in cerebrospinal fluid.[16] OGG1 can degrade 8-oxoG, reduce its damage on DNA, and thus reduce the damage of oxidative stress on brain cells.[17] OGG1 begins to decline in activity at the MCI stage, which promotes the progression of MCI to AD.[18] However, there are relatively few studies on whether the above gene polymorphisms are associated with MCI.

Epigenetics can modify aging and environmental factors.[19] DNA methylation is a major component of epigenetics involved in the pathophysiological processes of neurodegenerative diseases such as AD, other types of dementia, and cognitive decline.[20] Due to its relative stability and its ability to be directly regulated by underlying genetic sequences and environmental exposure, DNA methylation is thought to be a biomarker for brain-related diseases or disorders.[21] MCI is a precursor stage of AD, and cognitive function has been damaged to varying degrees. Its pathogenesis is affected by genetics and environment.[22] DNA methylation may play an important role in the development of MCI.[23] Our previous studies also found that DNA methylation of genes were associated with MCI.[24,25]

The ancestors of the Xinjiang Uygur population are Hui, and their bloodlines are mixed with Mongolian races and European races.[26] Therefore, the Chinese Uyghur bloodline composition is diversified, and there is a large genetic difference with the Chinese Han population. Moreover, the geographical environment, life and eating habits of Xinjiang in China are quite different from those in the inland areas of China, which may have an impact on the occurrence and development of diseases. Therefore, it is necessary to conduct genetic research in the Chinese Uyghur population.

Therefore, in order to further explore the relationship between the above genetic polymorphisms and MCI, this study examined the relationship of five gene polymorphisms (*OGG1* rs1052133, *BIN1* rs744373, *SORL1* rs1133174, *PSEN2* rs8383 and *NGF* rs6330) and promoter methylation of *OGG1* and dihydrolipoamide S-succinyltransferase (*DLST*) with MCI in a Xinjiang Uygur population. Our study provides a valuable evaluation of the role of these AD-related genes in MCI in this unique ethnic population.

MATERIALS AND METHODS

Samples and clinical data

One hundred and sixty-eight Uygur participants aged between 50 and 95 years (43 MCI, 125 cognitively normal) were selected at epidemiological surveys in 2015 in Hotan, Xinjiang. The patients' detailed characteristics are shown in Table 1. The epidemiology and related investigation were approved by the First Affiliated Hospital of Xinjiang Medical University Ethics Committee. All participants have provided their written informed consent.

All participants received neuropsychological tests to assess the level of cognition. Neuropsychological tests included: the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment Form (MoCA), Activities of Daily Living (ADLs) Scale, the overall Deterioration Scale (GDS), the Clinical

Psychogeriatrics published by John Wiley & Sons Australia, Ltd on behalf of Japanese Psychogeriatric Society

Table 1 The baseline clinical data of the included subjects

Characteristics	MCI N = 43 Mean ± SD	Control N = 125 Mean ± SD	Test value	P
Age (year)	71.56 ± 9.32	71.77 ± 8.16	t = −0.14	0.889
SBP (mmHg)	147.65 ± 29.20	140.52 ± 25.79	t = 1.51	0.133
DBP (mmHg)	79.93 ± 15.50	76.11 ± 15.91	Z = −1.46	0.143
TC (mmol/L)	4.98 ± 1.02	4.80 ± 1.21	t = 0.87	0.383
TG (mmol/L)	1.69 ± 1.01	1.83 ± 1.19	Z = −0.83	0.406
HDL (mmol/L)	1.50 ± 0.32	1.49 ± 0.59	Z = −0.69	0.493
LDL (mmol/L)	3.46 ± 0.88	3.14 ± 1.01	t = 1.90	0.059
GLU (mmol/L)	4.77 ± 1.44	4.63 ± 1.10	Z = −0.13	0.897
Gender	*n* (%)	*n* (%)		
Male	22 (51.2%)	65 (52.0%)	χ^2 = 0.009	0.924
Female	21 (48.8%)	60 (48.0%)		
Diabetes				
Yes	1 (2.3%)	1 (0.8%)	—	0.448
No	42 (97.7%)	124 (99.2%)		
Hypertension				
Yes	20 (46.5%)	51 (40.8%)	χ^2 = 0.428	0.513
No	23 (53.5%)	74 (59.2%)		
Cerebral vascular disease				
Yes	5 (11.6%)	9 (7.2%)	—	0.353
No	38 (88.4%)	116 (92.8%)		
Smoke				
No	38 (88.4%)	110 (88%)	χ^2 = 0.004	0.948
Yes	5 (11.6%)	15 (12%)		
Drink				
No	43 (100%%)	0 (0.0%)	—	1
Yes	124 (99.2%)	1 (0.8%)		
APOE ε4 carrier	7 (18.4%)	31 (81.6%)	χ^2 = 1.050	0.306
APOE non-ε4 carrier	33 (26.6.4%)	91 (73.4%)		

MCI, mild, cognitive impairment; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GLU, glucose; *APOE*, apolipoprotein E

Dementia Rating (CDR), and Hachinski ischaemic score (HIS) screening. Diagnosis criteria: a clinical diagnosis of AD was established according to the criteria of the Diagnostic and Statistical Manual-IV (DSM-IV).[27] Exclusion criteria for the current study were: (i) those with mental illness; (ii) any brain dysfunction that can cause neurological diseases such as cerebral haemorrhage, cerebral infarction, Parkinson's disease (PD), intracranial tumours; (iii) depression; and (iv) patients with severe cardiopulmonary liver and kidney dysfunction, severe infectious diseases, severe endocrine disease patients and toxic encephalopathy patients.

The blood biochemical indicators (including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), glucose (GLU)) were detected by an automatic biochemical analyzer (Beckman Coulter, Brea, CA, USA) at the Medical Testing Center of the First Affiliated Hospital of Xinjiang Medical University. Blood pressure (including systolic blood pressure (SBP), diastolic blood pressure (DBP)) was measured using a uniform sphygmomanometer (Omron Corporation, Kyoto, Japan).

DNA preparation, genotyping and methylation assay

Whole blood specimens were placed in tubes containing EDTA and stored at −80°C. Genomic DNA was extracted and dissolved in Tris-EDTA buffer, and then it was stored at −20°C. Polymerase chain reaction (PCR) was carried out in 40 μL containing 2 μL of each primer, 4 μL genomic DNA, 12 μL ddH_2O and 20 μL 2X HotTaq Master Mix. PCR was performed in a Veriti 96-well thermal cycler (Applied Biosystems, Foster City, CA, USA).The reverse primers of PCR consisted of an initial melting step at 95°C for 10 min, 35 cycles (*NGF*, *BIN1*, and *OGG1*) or 37 cycles (*PSEN2*) or 40 cycles (*SORL1*), and a final extension step at 72°C for 2 min. The

Table 2 Primers used for single nucleotide polymorphism analysis

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	°C
PSEN2 (rs8383)	TTACTTCTCCACGGACAAC	CAAGATTCTAACAGGACTCATC	55.3
BIN1 (rs744373)	GCCAGTCCATCTTCTTCT	ACCACATCTTAGCCACAG	57.6
NGF (rs6330)	CATCCATACTGCCTGAGTC	CCTGTGAGTCCTGTTGAAG	57.3
OGG1 (rs1052133)	GTGGATTCTCATTGCCTTC	AAACTGACTGCTTGATTTGG	57.5
SORL1 (rs1133174)	TGTGACTTGTGCTGTATGAT	ACGCTAGAAGAAGGCTTATC	51.5
OGG1 (methylation)	CGGTGGTTGAGTTTTATTTTC	CTCCTTACGACTTATCTTCTC	56.1
DLST (methylation)	GTTGTAGTCGGGATATTGG	CGAAACGAACCACTAACA	53.3

cycling program was 95°C for 30 s, 58°C (*NGF* and *BIN1*) or 54°C (*OGG1*) or 57°C (*PSEN2*) or 53°C (*SORL1*) for 45 s for annealing, and 72°C for 30 s. The details for the primer sequences were shown in Table 2. Genotyping was done using Sanger sequencing, gel electrophoresis and sequencing validation as shown in Figure 1. DNA bisulphite conversion was done using the EZ DNA Methylation-Gold™ Kit (ZYMO RESEARCH, Orange County, CA, USA). Promoter methylation status of *OGG1* and *DLST* were examined utilizing quantitative methylation-specific PCR (qMSP). Primer sequences of *OGG1* and *DLST* qMSP are shown in Table 3.

Statistical analysis

SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software was used for the statistical analysis. Comparison of demographical parameters between cases and controls was performed using Student's *t*-test for continuous variables and the χ^2 test for categorical data. Spearman rank correlation test was used to analyze the associations between gene methylation and metabolic characteristics of MCI subjects. The

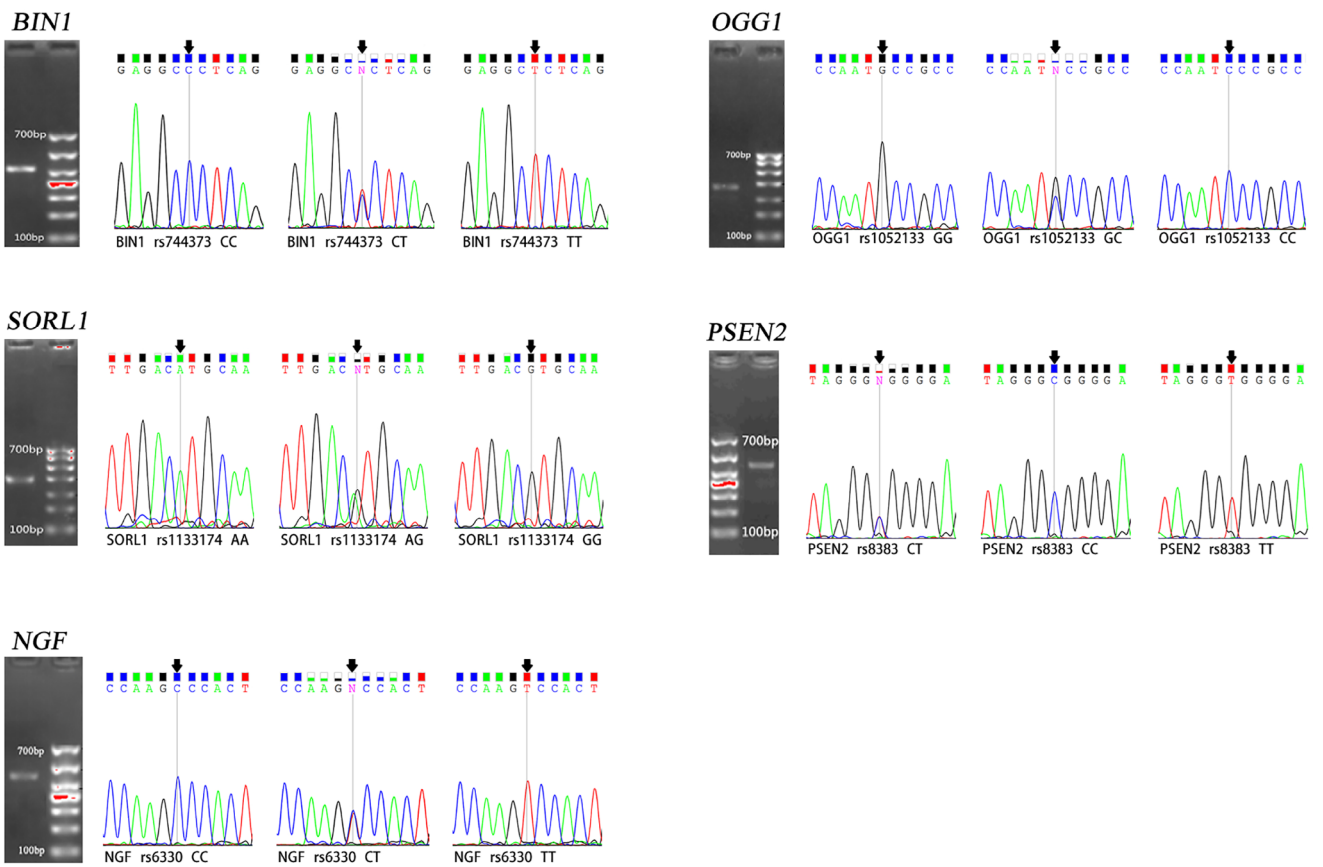

Figure 1 Representative results of gel electrophoresis and sequencing validation.

generalized multi-factor dimensionality reduction (GMDR) method was used to study the effects of gene-gene interactions and gene-environment interactions on the pathogenesis of MCI. GMDR detects and characterizes the nonlinear interactions between genetic and environmental factors. $P < 0.05$ was considered statistically significant.

RESULTS

General comparisons of the MCI group and control group in this study involved gender, age, hypertension, diabetes, blood lipids (TG, TC, HDL, LDL), smoking and drinking status (Table 1). Our results indicated there was no significant difference in the above phenotypes between the two groups ($P > 0.05$).

In this study, the genotype and allele frequencies of the five single nucleotide polymorphisms (SNPs) were consistent with the Hardy-Weinberg test in the control and sub-grouped control. We found no significant difference in genotype and allele frequency distribution between the MCI group and the control group ($P > 0.05$, Table 3), and no significant difference in dominant model and recessive model ($P > 0.05$, Supplementary Tables 1 and 2). Further subgroup tests by gender, apolipoprotein E (*APOE*) ε4 and APOE protein phenotypes showed no significant association of the five SNPs with MCI ($P > 0.05$, Tables 4 and 5).

In addition, this study compared the differences in methylation levels of the *DLST* and *OGG1* promoter regions in the MCI group and the control group

Table 3 Distribution frequencies of genotypes in mild cognitive impairment (MCI) cases and controls

Single nucleotide polymorphisms	MCI; Control (MM/Mm/mm)	*P* Genotype	MCI; Control (M/m)	*P* Allele	OR (95%CI)
Total					
BIN1 rs744373 (T > C)	20/22/1; 70/47/8	0.266	62/24; 187/63	0.621	0.870 (0.502–1.510)
NGF rs6330 (C > T)	22/20/1; 65/55/5	0.950	64/22; 185/65	0.939	1.022 (0.583–1.791)
OGG1 rs1052133 (C > G)	13/19/11; 40/62/23	0.594	45/41; 142/108	0.471	0.835 (0.511–1.365)
PSEN2 rs8383 (C > T)	17/19/7; 43/56/26	0.751	53/33; 142/108	0.434	1.222 (0.740–2.017)
SORL1 rs1133174 (A > G)	16/22/5; 50/63/12	0.872	54/32; 163/87	0.687	0.901 (0.541–1.498)
Males					
BIN1 rs744373 (T > C)	11/10/1; 35/26/4	0.919	32/12; 96/34	0.884	0.944 (0.437–2.040)
NGF rs6330 (C > T)	12/10/0; 34/29/2	1.000	34/10; 97/33	0.724	1.157 (0.516–2.595)
OGG1 rs1052133 (C > G)	8/8/6; 25/29/11	0.553	24/20; 79/51	0.468	0.775 (0.389–1.544)
PSEN2 rs8383 (C > T)	8/9/5; 21/30/14	0.908	25/19; 72/58	0.869	1.060 (0.532–2.112)
SORL1 rs1133174 (A > G)	6/13/3; 25/32/8	0.635	25/19; 82/48	0.461	0.770 (0.384–1.543)
Females					
BIN1 rs744373 (T > C)	9/12/0; 35/21/4	0.180	30/12; 91/29	0.572	0.797 (0.362–1.754)
NGF rs6330 (C > T)	10/10/1; 31/26/3	0.913	30/12; 88/32	0.811	0.909 (0.416–1.988)
OGG1 rs1052133 (C > G)	5/11/5; 15/33/12	0.934	21/21; 63/57	0.780	0.905 (0.448–1.827)
PSEN2 rs8383 (C > T)	9/10/2; 22/26/12	0.547	28/14; 70/50	0.342	1.429 (0.684–2.985)
SORL1 rs1133174 (A > G)	10/9/2; 25/31/4	0.676	29/13; 81/39	0.853	1.074 (0.504–2.291)

Table 4 Distribution frequencies of genotypes and alleles of subgroup analysis based on apolipoprotein E (APOE) ε4 allele in mild cognitive impairment (MCI) cases and controls

Single nucleotide polymorphisms	MCI; Control (MM/Mm/mm)	*P* Genotype	MCI; Control (M/m)	*P* Allele	OR (95%CI)
APOE ε4+					
BIN1 rs744373 (T > C)	4/3/0; 19/9/3	0.824	11/3; 47/15	1.000	1.170 (0.288–4.758)
NGF rs6330 (C > T)	1/5/1; 12/13/6	0.424	7/7; 37/25	0.508	0.676 (0.211–2.164)
OGG1 rs1052133 (C > G)	4/2/1; 7/20/4	0.179	10/4; 34/28	0.256	2.059 (0.582–7.279)
PSEN2 rs8383 (C > T)	4/3/0; 18/12/1	1.000	11/3; 48/14	1.000	1.069 (0.261–4.374)
SORL1 rs1133174 (A > G)	2/4/1; 13/15/3	0.721	8/6; 41/21	0.549	0.683 (0.209–2.227)
APOE ε4-					
BIN1 rs744373 (T > C)	15/17/1; 50/36/5	0.537	47/19; 136/46	0.578	0.837 (0.446–1.569)
NGF rs6330 (C > T)	15/14/4; 30/41/20	0.318	44/22; 101/81	0.115	1.604 (0.890–2.892)
OGG1 rs1052133 (C > G)	8/16/9; 30/42/19	0.586	32/34; 102/80	0.291	0.738 (0.420–1.298)
PSEN2 rs8383 (C > T)	17/15/1; 47/40/4	1.000	49/17; 134/48	0.922	1.032 (0.543–1.963)
SORL1 rs1133174 (A > G)	12/17/4; 36/46/9	0.837	39/25; 118/64	0.577	0.846 (0.470–1.522)

Table 5 Distribution frequencies of genotypes and alleles of subgroup analysis based on apolipoprotein E (APOE) gene protein phenotype in mild cognitive impairment (MCI) cases and controls

Single nucleotide polymorphisms	MCI; Control (MM/Mm/mm)	*p* Genotype	MCI; Control (M/m)	*p* Allele	OR (95%CI)
ApoE E2					
BIN1 rs744373 (T > C)	3/2/0; 12/4/1	0.689	8/2; 28/6	1.000	0.857 (0.144–5.097)
NGF rs6330 (C > T)	4/1/0; 8/9/0	0.470	9/1; 25/9	0.411	3.240 (0.358–29.299)
OGG1 rs1052133 (C > G)	1/1/3; 4/11/2	0.060	3/7; 19/15	0.150	0.338 (0.075–1.535)
PSEN2 rs8383 (C > T)	1/4/0; 6/6/5	0.240	6/4; 18/16	0.734	1.333 (0.318–5.590)
SORL1 rs1133174 (A > G)	1/4/0; 9/8/0	0.323	6/4; 26/8	0.422	0.462 (0.104–2.054)
ApoE E3					
BIN1 rs744373 (T > C)	13/17/1; 43/33/5	0.472	43/19; 119/43	0.539	0.818 (0.430–1.555)
NGF rs6330 (C > T)	14/16/1; 41/36/4	0.872	44/18; 118/44	0.779	0.911 (0.477–1.743)
OGG1 rs1052133 (C > G)	8/16/7; 28/35/18	0.643	32/30; 91/71	0.539	0.832 (0.463–1.497)
PSEN2 rs8383 (C > T)	15/11/5; 27/37/17	0.704	41/21; 91/71	0.175	1.523 (0.827–2.805)
SORL1 rs1133174 (A > G)	13/14/4; 33/39/9	0.945	40/22; 105/57	0.967	0.987 (0.535–1.820)
ApoE E4					
BIN1 rs744373 (T > C)	4/3/0; 15/10/2	1.000	11/3; 40/14	1.000	1.283 (0.312–5.279)
NGF rs6330 (C > T)	4/3/0; 16/10/1	1.000	11/3; 42/12	1.000	1.048 (0.251–4.372)
OGG1 rs1052133 (C > G)	4/2/1; 8/16/3	0.274	10/4; 32/22	0.404	1.719 (0.478–6.184)
PSEN2 rs8383 (C > T)	1/4/2; 10/13/4	0.549	6/8; 33/21	0.218	0.477 (0.145–1.571)
SORL1 rs1133174 (A > G)	2/4/1; 8/16/3	1.000	8/6; 32/22	0.886	0.917 (0.279–3.012)

(Fig. 2). The results showed that the methylation levels of *OGG1* and *DLST* genes were not significantly different between the two groups ($P > 0.05$).

DLST methylation in female controls was significantly lower than that in male controls (Fig. 2, $P = 0.003$). In the *APOE* ε4 subgroup, *DLST* methylation was significantly lower in MCI (Fig. 2, $P = 0.042$). in the non-*APOE* ε4 subgroup, *DLST* methylation in the male controls was significantly lower than that in the female controls (Fig. 2, $P = 0.04$). In the non-*APOE* ε4 carrier younger than 75, *OGG1* methylation was significantly increased in MCI (Fig. 2, $P = 0.049$).

We also analyzed the correlation of *DLST* and *OGG1* gene methylation levels with clinical phenotypes. Our results showed there was no significant positive correlation between the methylation levels of *OGG1* and *DLST* genes and age ($P > 0.05$, data not shown). In the MCI group, *DLST* methylation levels were inversely correlated with LDL (Table 6, $r = -0.311$, $P = 0.048$). Further analysis by sex showed there was a positive correlation of FBG and LDL with *OGG1* methylation levels in the female controls (Table 6, FBG: $r = 0.294$, $P = 0.024$; HDL: $r = 0.278$, $P = 0.033$). There was a significant inverse correlation between LDL and *DLST* methylation levels in the male MCI group (Table 6, $r = -0.455$, $P = 0.033$). HDL was positively correlated with *DLST* methylation levels in the female control group (Table 6, $r = 0.492$, $P = 0.000$).

Based on the above results, we further analyzed the interaction of SNPs of five genes. Our results showed that the best model was *OGG1* methylation – *BIN1* rs744373 – *OGG1* rs1052133 – *PSEN2* rs8383 – *APOE* rs7412 rs429358 (Table 7, $P = 0.001$), indicating that the interaction of *OGG1* promoter methylation with several other factors increased the risk of MCI.

DISCUSSION

The neuropathological changes in MCI partially overlap with those in AD. For example, neurofibrillary tangles (NFT) and neuritic plaques in the neocortex of the temporal lobe of AD patients can also be seen in MCI patients.[28] Oxidative DNA damage was also found to be significantly increased in brain tissue and peripheral blood lymphocytes of MCI patients,[29–31] which is consistent with DNA damage caused by oxidative stress (reactive oxygen species (ROS)) in the AD brains.[32] Therefore, we studied the methylation levels of *DLST* and *OGG1*, which are closely related to ROS.

DLST is one of the three protein subunits of α-ketoglutarate dehydrogenase complex (KGDHC), and it is the major subunit that affects KGDHC activity.[33] KGDHC is a rate-limiting enzyme that mediates the oxidative decarboxylation of α-ketoglutarate in the tricarboxylic acid (TCA) cycle. Its decreased activity leads to a decrease in glucose metabolism

Psychogeriatrics published by John Wiley & Sons Australia, Ltd on behalf of Japanese Psychogeriatric Society

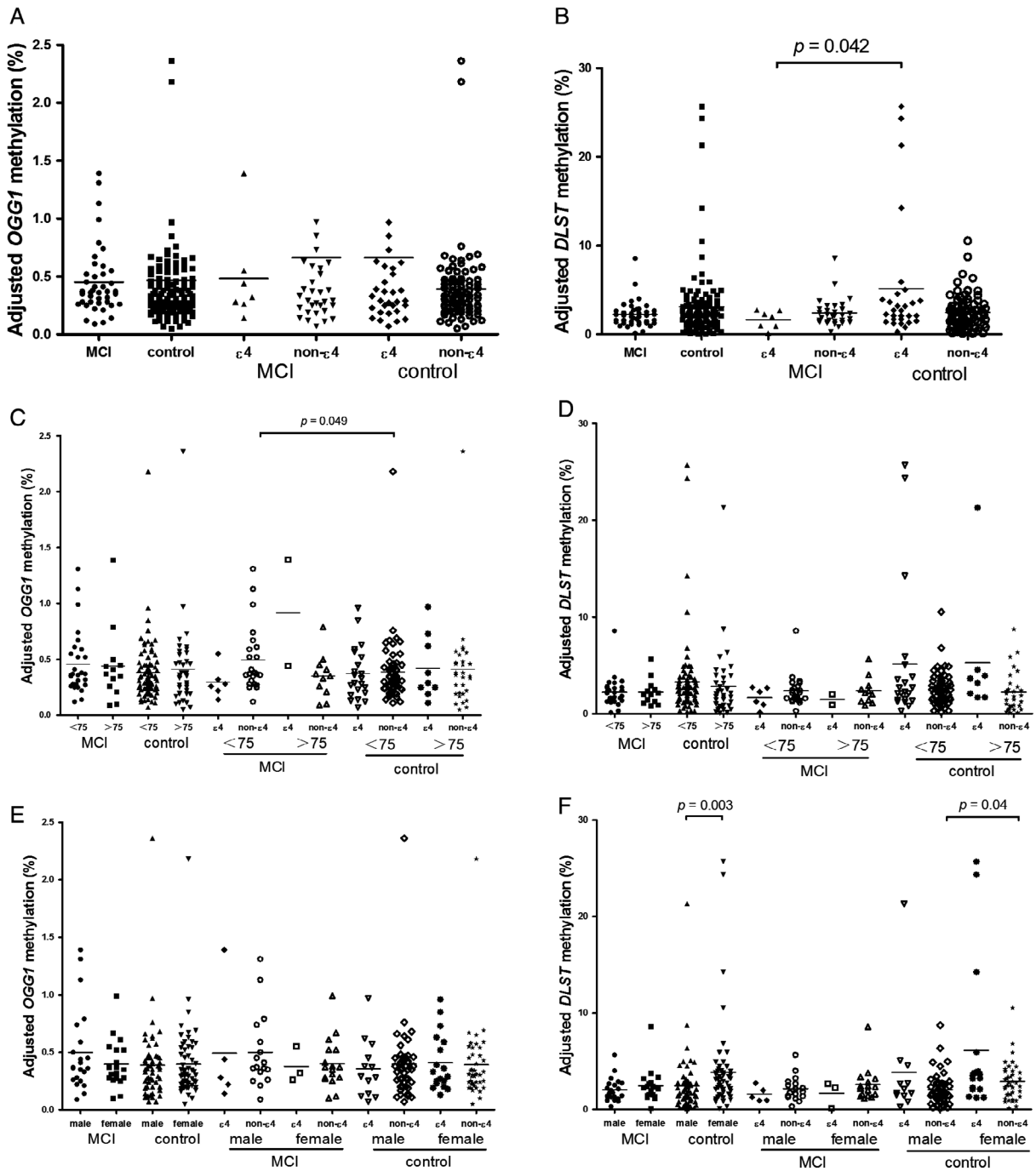

Figure 2 Association of 8-oxoguanine DNA glycosylase 1 (*OGG1*) and dihydrolipoamide S-succinyltransferase (*DLST*) methylation levels with mild cognitive impairment (MCI) in the total and subgroup samples stratified by gender, age, and apolipoprotein E (*APOE*) ε4 (A-F).

in the brain, which in turn affects cognitive function.[34] Since abnormal glucose metabolism in the brain is a common feature of dementia and can occur several decades before the clinical symptoms of AD,[35–37] the *DLST* gene is one of the important candidate genes affecting cognitive function. In the present study, we found that the hypomethylation level of the *DLST* promoter interacted with *APOE* ε4 and was associated with the risk of MCI. We also observed that LDL might affect *DLST* promoter

Table 6 Correlation tests between genes (OGG1 and DLST) methylation level and important parameters

	OGG1				*DLST*			
	MCI		Control		MCI		Control	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Total								
FBG	0.25	0.11	0.111	0.231	−0.061	0.704	0.167	0.074
TG	−0.203	0.196	−0.052	0.573	0.252	0.112	0.134	0.151
TC	0.011	0.943	−0.04	0.668	−0.208	0.192	−0.049	0.603
HDL	0.038	0.81	0.096	0.3	−0.229	0.149	−0.052	0.579
LDL	0.009	0.956	0.022	0.815	−0.311	**0.048**	0.006	0.953
Female								
FBG	0.233	0.323	0.294	**0.024**	0.139	0.571	0.067	0.619
TG	−0.338	0.145	−0.038	0.776	0.33	0.167	−0.07	0.601
TC	0.145	0.543	−0.034	0.8	−0.123	0.616	0.006	0.963
HDL	0.386	0.093	0.278	**0.033**	−0.404	0.086	0.492	**0**
LDL	0.072	0.764	0.037	0.78	−0.209	0.391	0.088	0.51
Male								
FBG	0.302	0.172	0.015	0.912	−0.36	0.1	0.105	0.431
TG	−0.039	0.865	−0.086	0.518	−0.065	0.774	0.137	0.306
TC	−0.02	0.931	0.067	0.616	−0.364	0.096	−0.065	0.628
HDL	−0.149	0.507	0.074	0.578	−0.005	0.982	−0.044	0.743
LDL	−0.014	0.951	0.066	0.622	−0.455	**0.033**	−0.006	0.963

TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FBG, fasting plasma glucose; *OGG1*, 8-oxoguanine DNA glycosylase 1; *DLST*, dihydrolipoamide S-succinyltransferase; Bold, statistically significant

Table 7 Generalized multi-factor dimensionality reduction models of high-order interaction on mild cognitive impairment risk

Model	Training balance accuracy	Testing balance accuracy	Sign test (*P*)	Cross-validation consistency
OGG1 rs1052133 - *APOE* rs7412 rs429358	0.637	0.4774	5 (0.6230)	7/10
BIN1 rs744373 - *PSEN2* rs8383 - *APOE* rs7412 rs429358	0.7084	0.6363	8 (0.0547)	10/10
BIN1 rs744373 - *OGG1* rs1052133 - *PSEN2* rs8383 - *APOE* rs7412 rs429358	0.7976	0.5462	7 (0.1719)	10/10
BIN1 rs744373 - ***NGF* rs6330** - *OGG1* rs1052133 - *PSEN2* rs8383 - *APOE* rs7412 rs429358	0.8741	0.6461	**9 (0.0107)**	9/10
***DLST* methylation** - *BIN1* rs744373 - *OGG1* rs1052133 - *PSEN2* rs8383 - *APOE* rs7412 rs429358	0.8621	0.5962	**9 (0.0107)**	10/10
***OGG1* methylation** - *BIN1* rs744373 - *OGG1* rs1052133 - *PSEN2* rs8383 - *APOE* rs7412 rs429358	0.8767	0.6395	**10 (0.0010)**	8/10
***DLST* methylation - *OGG1* methylation** - *BIN1* rs744373 - *OGG1* rs1052133 - *PSEN2* rs8383 - *APOE* rs7412 rs429358	0.9271	0.595	8 (0.0547)	10/10
***DLST* methylation - *OGG1* methylation** - *BIN1* rs744373 - ***NGF* rs6330** - *OGG1* rs1052133 - *PSEN2* rs8383 - *APOE* rs7412 rs429358	0.9538	0.5425	5 (0.6230)	10/10

Bold, statistically significant

methylation and promote the pathogenesis of MCI in males.

OGG1 degrades 8-oxoG to reduce its damage to DNA bases.[17] In MCI brains, the 8-oxoG content was significantly increased, and the activity of OGG1 was significantly decreased.[18] It has been found that *OGG1* gene polymorphism mutations can alter OGG1 catalytic activity and cause DNA damage, eventually leading to cognitive impairment.[17] *OGG1* hypermethylation has been found to be significantly associated with aging in mice.[38] Our study found that the hypermethylation level of the *OGG1* promoter may

Psychogeriatrics published by John Wiley & Sons Australia, Ltd on behalf of Japanese Psychogeriatric Society

increase the risk of MCI in the non-*APOE* ε4 carriers under 75 years of age. Our findings provided a molecular basis for further study of the role of DNA damage in the pathogenesis of MCI.

However, our study did not find that *BIN1* rs744373, *SORL* rs1133174, *PSEN2* rs8383, *NGF* rs6330, *OGG1* rs1052133 polymorphisms were associated with MCI in Chinese Uygur. This might also be due to a moderate sample size in the current study. Therefore, whether the above gene loci are related to MCI and whether there are ethnic differences need to be studied with larger sample sizes.

To further investigate whether DNA methylation interacts with SNPs, we interacted *DLST* and *OGG1* methylation with the five polymorphisms. Our results indicated that the best model was *OGG1* methylation – *BIN1* rs744373 – *OGG1* rs1052133 – *PSEN2* rs8383 – *APOE* rs7412 rs429358, indicating that the interaction of *OGG1* promoter methylation with several other factors might increase the risk of MCI.

The results of genetic association in this study differ from other research conclusions. Analysis of lifestyle, genetic background, cultural differences, and geographical differences may be the main reasons. Second, the sample size is limited. A larger sample size is needed later to verify our results. Third, older subjects usually have more underlying diseases. Although we have attempted to control confounding factors, unknown influencing factors might still exist. It is necessary to further expand the sample size and verify our findings in other ethnic groups.

This study found for the first time that *DLST* promoter methylation interacts with *APOE* ε4, which affects the pathogenesis of MCI. In addition, *OGG1* promoter methylation interacts with several other factors to increase the risk of MCI.

ACKNOWLEDGMENT

We are grateful to staff who joined in the epidemiological survey in 2015 in Hotan Region, Xinjiang province, China. This research was supported by grants from the National Natural Science Foundation of China (No. 81360064) and the High Technology Research and Development Projects of Xinjiang Province (No. 201517104), and K. C. Wong Magna Fund in Ningbo University.

REFERENCES

1 Korolev IO, Symonds LL, Bozoki AC. Predicting progression from mild cognitive impairment to Alzheimer's dementia using clinical, MRI, and plasma biomarkers via probabilistic pattern classification. *PLoS One* 2016; **11**: e0138866.
2 Luck T, Luppa M, Briel S, Riedel-Heller SG. Incidence of mild cognitive impairment: a systematic review. *Dement Geriatr Cogn Disord* 2010; **29**: 164–175.
3 Geda YE, Roberts RO, Mielke MM *et al*. Baseline neuropsychiatric symptoms and the risk of incident mild cognitive impairment: a population-based study. *Am J Psychiatry* 2014; **171**: 572–581.
4 Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. *Acta Psychiatr Scand* 2009; **119**: 252–265.
5 Shi YM, Zhou H, Zhang ZJ *et al*. Association of the LRP1 gene and cognitive performance with amnestic mild cognitive impairment in elderly Chinese. *Int Psychogeriatr* 2009; **21**: 1072–1080.
6 Jin C, Zhang L, Xian Y *et al*. The SORL1 polymorphism rs985421 may confer the risk for amnestic mild cognitive impairment and Alzheimer's disease in the Han Chinese population. *Neurosci Lett* 2014; **563**: 80–84.
7 Gao X, Liu M, Sun L *et al*. SORL1 genetic variants modulate risk of amnestic mild cognitive impairment in northern Han Chinese. *Int J Neurosci* 2014; **124**: 296–301.
8 Gamarra D, Elcoroaristizabal X, Fernandez-Martinez M, de Pancorbo MM. Association of the C47T polymorphism in SOD2 with amnestic mild cognitive impairment and Alzheimer's disease in carriers of the APOEepsilon4 allele. *Dis Markers* 2015; **2015**: 746329.
9 Tan MS, Yu JT, Tan L. Bridging integrator 1 (BIN1): form, function, and Alzheimer's disease. *Trends Mol Med* 2013; **19**: 594–603.
10 Chou CT, Liao YC, Lee WJ, Wang SJ, Fuh JL. SORL1 gene, plasma biomarkers, and the risk of Alzheimer's disease for the Han Chinese population in Taiwan. *Alzheimers Res Ther* 2016; **8**: 53.
11 Guo LH, Westerteicher C, Wang XH *et al*. SORL1 genetic variants and cerebrospinal fluid biomarkers of Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci* 2012; **262**: 529–534.
12 Alexopoulos P, Guo LH, Kratzer M, Westerteicher C, Kurz A, Perneczky R. Impact of SORL1 single nucleotide polymorphisms on Alzheimer's disease cerebrospinal fluid markers. *Dement Geriatr Cogn Disord* 2011; **32**: 164–170.
13 Kolsch H, Jessen F, Wiltfang J *et al*. Influence of SORL1 gene variants: association with CSF amyloid-beta products in probable Alzheimer's disease. *Neurosci Lett* 2008; **440**: 68–71.
14 Sarasija S, Laboy JT, Ashkavand Z, Bonner J, Tang Y, Norman KR. Presenilin mutations deregulate mitochondrial Ca(2+) homeostasis and metabolic activity causing neurodegeneration in *Caenorhabditis elegans*. *Elife* 2018; **7**: e33052.
15 Parikh V, Howe WM, Welchko RM *et al*. Diminished trkA receptor signaling reveals cholinergic-attentional vulnerability of aging. *Eur J Neurosci* 2013; **37**: 278–293.
16 Ferreira D, Westman E, Eyjolfsdottir H *et al*. Brain changes in Alzheimer's disease patients with implanted encapsulated cells releasing nerve growth factor. *J Alzheimers Dis* 2015; **43**: 1059–1072.
17 Jacob KD, Noren Hooten N, Tadokoro T, Lohani A, Barnes J, Evans MK. Alzheimer's disease-associated polymorphisms in human OGG1 alter catalytic activity and sensitize cells to DNA damage. *Free Radic Biol Med* 2013; **63**: 115–125.

18 Shao C, Xiong S, Li GM *et al*. Altered 8-oxoguanine glycosylase in mild cognitive impairment and late-stage Alzheimer's disease brain. *Free Radic Biol Med* 2008; **45**: 813–819.
19 Yang N, Wei Y, Xu Q, Tang B. [Progress in epigenetic research on Alzheimer disease]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2016; **33**: 252–255.
20 Delgado-Morales R, Esteller M. Opening up the DNA methylome of dementia. *Mol Psychiatry* 2017; **22**: 485–496.
21 van den Hove DL, Kenis G, Rutten BP. Epigenetic dysregulation in Alzheimer's disease: cause or consequence? *Epigenomics* 2014; **6**: 9–11.
22 Young JW, Sharkey J, Finlayson K. Progressive impairment in olfactory working memory in a mouse model of mild cognitive impairment. *Neurobiol Aging* 2009; **30**: 1430–1443.
23 van Bergen JM, Li X, Hua J *et al*. Colocalization of cerebral iron with amyloid beta in mild cognitive impairment. *Sci Rep* 2016; **6**: 35514.
24 Luo M, Zhou X, Ji H *et al*. Population difference in the associations of KLOTH promoter methylation with mild cognitive impairment in Xinjiang Uygur and Han populations. *PLoS One* 2015; **10**: e0132156.
25 Liu G, Ji H, Liu J *et al*. Association of OPRK1 and OPRM1 methylation with mild cognitive impairment in Xinjiang Han and Uygur populations. *Neurosci Lett* 2017; **636**: 170–176.
26 Bin W, Shengmin Y. Uygur ethnic composition and evolution based on incidences of typical Mongoloid physical characteristics. *Acta Anthropologica Sinica* 2017; **36**: 227–235.
27 AP Association. *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV*, 4th edn. Washington, DC: American Psychiatric Association, 1994.
28 Markesbery WR, Schmitt FA, Kryscio RJ, Davis DG, Smith CD, Wekstein DR. Neuropathologic substrate of mild cognitive impairment. *Arch Neurol* 2006; **63**: 38–46.
29 Migliore L, Fontana I, Trippi F *et al*. Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. *Neurobiol Aging* 2005; **26**: 567–573.
30 Keller JN, Schmitt FA, Scheff SW *et al*. Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology* 2005; **64**: 1152–1156.
31 Lovell MA, Markesbery WR. Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. *Nucleic Acids Res* 2007; **35**: 7497–7504.
32 Chouliaras L, Mastroeni D, Delvaux E *et al*. Consistent decrease in global DNA methylation and hydroxymethylation in the hippocampus of Alzheimer's disease patients. *Neurobiol Aging* 2013; **34**: 2091–2099.
33 Nilsen LH, Shi Q, Gibson GE, Sonnewald U. Brain [U-13 C]glucose metabolism in mice with decreased alpha-ketoglutarate dehydrogenase complex activity. *J Neurosci Res* 2011; **89**: 1997–2007.
34 Bubber P, Haroutunian V, Fisch G, Blass JP, Gibson GE. Mitochondrial abnormalities in Alzheimer brain: mechanistic implications. *Ann Neurol* 2005; **57**: 695–703.
35 Small GW, Mazziotta JC, Collins MT *et al*. Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease. *JAMA* 1995; **273**: 942–947.
36 Reiman EM, Caselli RJ, Yun LS *et al*. Preclinical evidence of Alzheimer's disease in persons homozygous for the epsilon 4 allele for apolipoprotein E. *N Engl J Med* 1996; **334**: 752–758.
37 Mosconi L, De Santi S, Li J *et al*. Hippocampal hypometabolism predicts cognitive decline from normal aging. *Neurobiol Aging* 2008; **29**: 676–692.
38 Langie SA, Cameron KM, Ficz G *et al*. The ageing brain: effects on DNA repair and DNA methylation in mice. *Genes (Basel)* 2017; **8**: E75.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website: http://onlinelibrary.wiley.com/doi//suppinfo.

Supplementary Table 1 Distribution frequencies of dominant model and recessive model in mild cognitive impairment (MCI) cases and controls.

Supplementary Table 2 Distribution frequencies of dominant model and recessive model of subgroups analyzed based on *APOE* ε4 allele in mild cognitive impairment (MCI) cases and controls.

Psychogeriatrics published by John Wiley & Sons Australia, Ltd on behalf of Japanese Psychogeriatric Society