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Significant association of 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*) rs3846662 and sirtuin 1 (*SIRT1*) rs7895833 and apolipoprotein E (*APOE*) hypermethylation with mild cognitive impairment (MCI)

Ting Zou, PhD[a], Yali Duan, MSc[a], Xiaohui Zhou, PhD[a,*], Wei Chen, MSc[a], Xiuru Ying, MBBS[b], Guili Liu, MSc[b], Yongjie Zhao, MSc[a], Meisheng Zhu, MSc[a], Abuliz Pari, MSc[a], Kader Alimu, MSc[a], Haijun Miao, PhD[a], Keyim Kabinur, MD[a], Lei Zhang, MSc[a], Qinwen Wang, PhD[b,*], Shiwei Duan, PhD[b,*]

Abstract

Our study investigated the association of five genes with MCI in the Xinjiang Uygur population in China. In addition, we also analyzed the association between *APOE* methylation and MCI.

Forty-three MCI and 125 controls were included in the present study. Genotyping was done by Sanger sequencing. DNA methylation assay was done using quantitative methylation-specific polymerase chain reaction (qMSP).

The distribution of *HMGCR* rs3846662 allele frequencies was significantly different between the MCI group and the control group ($P = .04$), especially in women ($P = .032$). Subgroup analysis showed that there was a statistically significant association of *HMGCR* rs3846662 with MCI in the non-*APOE* ε4 group ($P = .024$), especially in the females with non-*APOE* ε4. Similarly, *HMGCR* rs3846662 genotype and allele frequency in the ApoE E2 protein group were significantly different in the MCI group and the control group (genotype $P = .021$; allele $P = .007$). In addition, *SIRT1* rs7895833 genotype frequency in the *APOE* ε4 group was found to be significantly different between the MCI and the control group ($P = .005$). We also observed a significant association of *SIRT1* rs7895833 with MCI in the ApoE E4 protein subgroup ($P = .005$). In addition, *APOE* methylation levels were significantly different between the MCI group and the control group ($P = .021$), especially in men ($P = .006$). Subgroup analysis showed that *APOE* methylation levels were significantly associated with MCI in the non-*APOE* ε4 group ($P = .009$), especially in men ($P = .015$).

This study found a significant association of *HMGCR* rs3846662 with MCI in females independent of *APOE* ε4. In contrast, we revealed that the association of *SIRT1* rs7895833 with MCI was dependent on with *APOE* ε4. We also showed that hypermethylation of *APOE* in MCI was independent of *APOE* ε4.

Abbreviations: AD = Alzheimer's disease, ADL = activities of daily living, APH1B = Aph-1 homolog B, APOE = apolipoprotein E, CDR = clinical dementia rating, GDS = global deterioration scale, HMGCR = 3-hydroxy-3-methylglutaryl-CoA reductase, KL = Klotho, MAFs = minor allele frequencies, MCI = mild cognitive impairment, MMSE = Mini-mental State Examination, PRNP = Prion protein, qMSP = quantitative methylation-specific polymerase chain reaction, SIRT = sirtuin.

Keywords: APOE, HMGCR, MCI, methylation, SIRT1

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[a] Department of Geriatrics, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang Province, [b] Ningbo Key Lab of Behavior Neuroscience, Zhejiang Provincial Key Laboratory of Pathophysiology, School of Medicine, Ningbo University, Ningbo, Zhejiang Province, China.

[*] Correspondence: Xiaohui Zhou, Department of Geriatrics, The First Affiliated Hospital of Xinjiang Medical University, No.393 Liyushan Road, Urumqi, Xinjiang Province (e-mail: zhouxiaohui858@sina.com); Qinwen Wang, Ningbo Key Lab of Behavior Neuroscience, School of Medicine, Ningbo University, No.818 Fenghua Road, Ningbo, Zhejiang Province (e-mail: wangqinwen@nbu.edu.cn); Shiwei Duan, Medical Genetics Center, School of Medicine, Ningbo University, No.818 Fenghua Road, Ningbo 315000, China (e-mail: duanshiwei@nbu.edu.cn).

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1. Introduction

Mild cognitive impairment (MCI) is a transitional stage between normal aging and Alzheimer's disease (AD),[1,2] with 10 to 20% MCI patients progressed to AD per year.[3,4] Therefore, studying the pathogenesis of MCI may help early prevention and treatment of AD.

The pathogenesis of MCI is complex and is influenced by genetic and environmental factors. *APOE* is one of the most studied genes in the cognitive field. *APOE* ε4 carriers are prone to Aβ deposition and Tau protein entanglement, which may increase the risk of MCI,[5,6] while *APOE* ε2 reduces the risk of MCI and AD.[7] Sirtuins (SIRTs) are a group of NAD-dependent deacetylases, in which SIRT1 has a significant delay in aging and neuroprotection,[8,9] and a significant decrease of serum SIRT1 concentration was found in MCI and AD patients.[10] Aph-1 homolog B (APH1B) is one of the subtypes of the γ-secretase protein complex, and it can specifically cleave the Aβ protein precursor (APP).[11–14] Genetic polymorphism of *APH1B* was found to affect the content of Aβ in the brain.[15] Prion protein (PRNP) has a high affinity with Aβ protein,[16] and previous study has shown that genetic polymorphism of *PRNP* was associated with cognitive decline in the elderly.[17] Elevated serum cholesterol increases the risk of AD.[18] 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGCR) is the rate-limiting enzyme in cholesterol synthesis, and HMGCR can bind to statins to inhibit cholesterol production. A number of studies have found that *HMGCR* gene polymorphisms are associated with cognitive function.[19,20] Recent studies have also found that *HMGCR* rs3846662 may participate in the pathophysiological process from MCI to AD by affecting glucose metabolism in the brain.[21]

DNA methylation is a major component of epigenetics, and its mechanism is through environmental factors affecting AD.[22] MCI is a precursor stage of AD. DNA methylation may play an important role in the development of MCI.[23] A recent study has found that *APOE* gene methylation levels in the brain of AD patients and normal people may be involved in the pathogenesis of AD,[24] but its relationship with MCI is still unclear.

The ancestors of the Xinjiang Uygur ethnic group in China are Huiwu people with complex genetic background. Moreover, the environmental factors such as geographical environment, life and eating habits in Xinjiang are quite different from those in the inland areas of China, which may have an impact on the occurrence and development of diseases. Our previous studies have found that the promoter methylation of Klotho (*KL*) is ethnically different among Chinese Uygur and Han populations in Xinjiang.[25,26]

Therefore, in order to further explore the genetic and environmental risk factors of MCI in Xinjiang Uygur, we studied the relationship of four genes (*APH1B*, *HMGCR*, *SIRT1*, *PRNP*) and MCI. Meanwhile, the differences in methylation of the *APOE* gene between MCI patients and normal controls were also compared to identify the genetic and epigenetic risk factors of MCI in Xinjiang Uygur population.

2. Materials and methods

2.1. Subjects

A total of 168 subjects were studied, including 43 in the MCI group and 125 in the control group. All of them were from the epidemiological survey of cognitive dysfunction in the Uygur elderly over 60 in the Hotan area of Xinjiang, China. The general conditions and clinical data of the two groups were compared in Table 1. All the enrolled subjects had signed the informed consent forms. And the present research was reviewed and approved by the Medical Research Ethics Committee of Xinjiang Medical University.

The diagnostic criteria of MCI referred to the MCI diagnostic criteria of the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, Revision 4 (DSM-IV). Specifically, the criteria are:

1. There is a memory complaint provided by the patient, family or acquaintance;
2. Objective evidence of memory loss (Memory test scores are 1.5 standard deviations lower than the normal standard of age and education level);
3. the score of global deterioration scale (GDS) is within the range of 2 and 3; the score of clinical dementia rating (CDR) is 0.5;
4. general cognitive function is normal;
5. the activities of daily living (ADL) remain normal;
6. Exclude other physical and mental disorders that cause dementia and any brain dysfunction.

And the exclusion criteria of MCI are:

1. exclude those with a history of mental illness or congenital mental retardation;

Table 1

The baseline clinical data of included subjects in Xinjiang Uygur participant.

Characteristics	Cases (n = 43)	Controls (n = 125)	χ^2/t	P
Male/female	22/21	65/60	0.009	1
Drinking (yes/no)	0/43	1/124	0.346	1
Smoking (yes/no)	5/38	15/110	0.004	.948
Hypertension (yes/no)	20/23	51/74	0.428	.513
Diabetes (yes/no)	1/42	1/124	0.633	.448
Age (years)	71.56 ± 9.32	71.77 ± 8.16	/	.733
Fasting blood glucose (FBG) (mmol/L)	4.77 ± 1.44	4.63 ± 1.10	/	.897
Triglyceride (TG) (mmol/L)	1.69 ± 1.01	1.83 ± 1.19	/	.406
Total cholesterol (TC) (mmol/L)	4.98 ± 1.00	4.79 ± 1.20	0.908	.365
High density lipoprotein (HDL) (mmol/L)	1.50 ± 0.32	1.49 ± 0.59	/	.493
Low density lipoprotein (LDL) (mmol/L)	3.46 ± 0.88	3.14 ± 1.01	1.898	.059
Systolic blood pressure (SBP) (mm Hg)	147.65 ± 29.20	140.52 ± 25.79	1.511	.133
Diastolic blood pressure (DBP) (mm Hg)	79.93 ± 15.50	76.11 ± 15.91	/	.143
MMSE	16.09 ± 2.55	24.80 ± 2.49	/	<.01

2. exclude patients with severe cardiopulmonary liver and kidney dysfunction, severe endocrine disease, severe infectious diseases and patients with toxic encephalopathy;
3. exclude neurological diseases that can cause brain dysfunction, such as stroke, Parkinson's disease, brain tumors, etc;
4. exclude vascular dementia (HIS ischemic index > 4 points);
5. exclude depression;
6. exclude those with a history of head trauma, history of taking special drugs, etc;
7. exclude those who have been identified as alcohol or drug dependent in the past 6 months.

The inclusion criteria of the control group are: the participants were selected from the same survey site, and the healthy people who had a life background, age, gender, ethnicity, and cultural level matched with the MCI group and had no kinship after strict examination.

2.2. DNA preparation, genotyping and methylation assay

All the subjects' fasting venous blood were extracted in the morning, added with EDTA anticoagulant and stored in a refrigerator at −80°C. We extracted DNA of blood sample using the blood genomic DNA extraction kit (Omega Bio-tek, Inc. USA). The concentration and purity of the extracted DNA were measured under an ND1000 ultra-micro UV spectrophotometer (Nanodrop1000, Wilmington, USA).

Primer sequences of the four polymorphisms (*APH1B* rs1047552, *SIRT1* rs7895833, *HMGCR* rs3846662, and *PRNP* rs1799990) were shown in Supplemental Table 1, http://links.lww.com/MD/D105. The 40 μL reaction system consists of 2 μL of upstream and downstream primers, 4 μL of DNA template, 12 μL of ddH_2O, and 20 μL of 2X HotTaq Master Mix. The PCR conditions were: pre-denaturation at 95°C for 10 min, denaturation at 95°C for 30 s, annealing for 45 s (see Supplemental Table 1, http://links.lww.com/MD/D105 for annealing temperatures), extension at 72°C for 30 s, 35 cycles, 72°C extension for 7 min, and termination at 10°C. Genotyping was performed using Sanger sequencing, and the electropherograms and sequencing results of the amplified fragments were shown in Fig. 1.

Bisulphite coversion was done using the EZ DNA Methylation-Gold™ Kit (Zymo Research, Orange County, CA, USA). The methylation of the *APOE* gene was detected by qMSP, and the primer sequence of qMSP was used (see Supplemental Table 1, http://links.lww.com/MD/D105), and finally verify the qMSP results by Sanger sequencing. Capillary electropherogram and sequencing were shown in Fig. 2b.

3. Results

As shown in Table 1, there was a significant difference of the MMSE scores between MCI group and control group ($P < .05$). In this study, the genotype frequencies of *SIRT1* rs7895833, *APH1B* rs1047552, *PRNP* rs1799990, *HMGCR* rs3846662 were consistent with Hardy-Weinberg equilibrium in both MCI group and control group.

The distribution of *HMGCR* rs3846662 allele frequencies was significantly different between the MCI group and the control group (Table 2, $P = .04$, OR = 1.709, 95% CI = 1.021 to2.860), especially in women (Table 2, $P = .032$, OR = 2.211, 95% CI = 1.060 to 4.609). Further subgroup analysis by *APOE* ε4 allele showed that *HMGCR* rs3846662 was statistically associated with MCI in the non-*APOE* ε4 subgroup (Table 3, $P = .024$, OR = 1.960, 95% CI = 1.089 to 3.528). Breakdown analysis showed that *HMGCR* rs3846662 was associated with MCI in the females with non-*APOE* ε4 (Table 3, genotype: $P = .036$; allele: $P = .021$, OR = 2.622, 95% CI = 1.145 to 6.006). We also performed subgroup analysis by ApoE protein types. And our results confirmed that the *HMGCR* rs3846662 genotype and allele frequency in the ApoE E2 protein group were significantly different between the MCI group and the control group (Table 4, genotype in total samples: $P = .021$; Allele: $P = .007$, OR = 0.615, 95% CI = 0.454 to 0.834; genotype in the female subgroup: $P = .063$; Allele: $P = .023$, OR = 2.000, 95% CI = 1.260 to 3.174). These results showed that the female-specific association of *HMGCR* rs3846662 with MCI was independent of *APOE* ε4.

In addition, the distribution of *SIRT1* rs7895833 genotype frequencies in the *APOE* ε4 group was statistically significant between MCI group and control group (Table 3, $P = .005$).

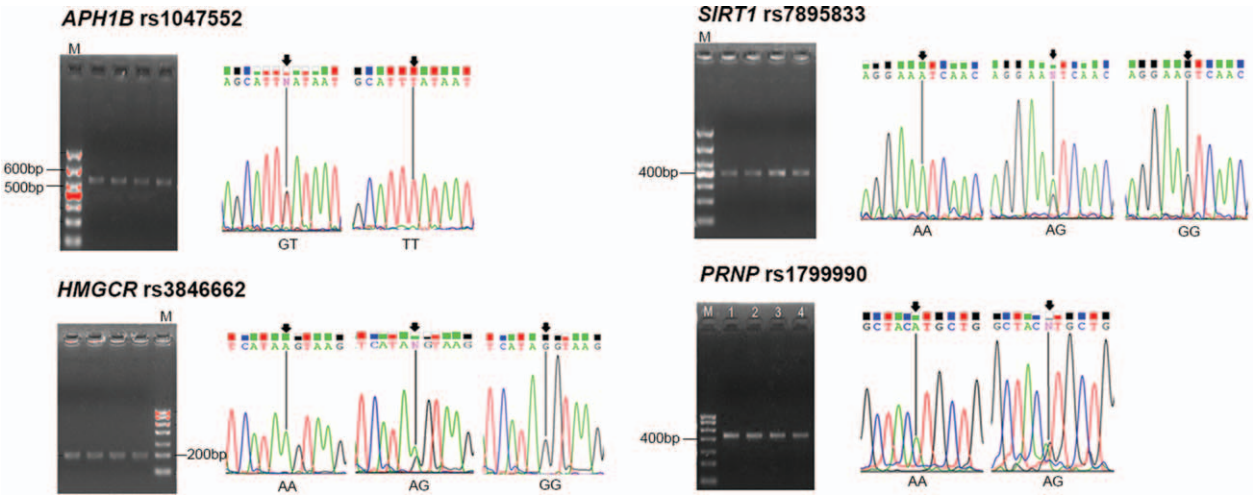

Figure 1. Representative results of gel electrophoresis and sequencing validation.

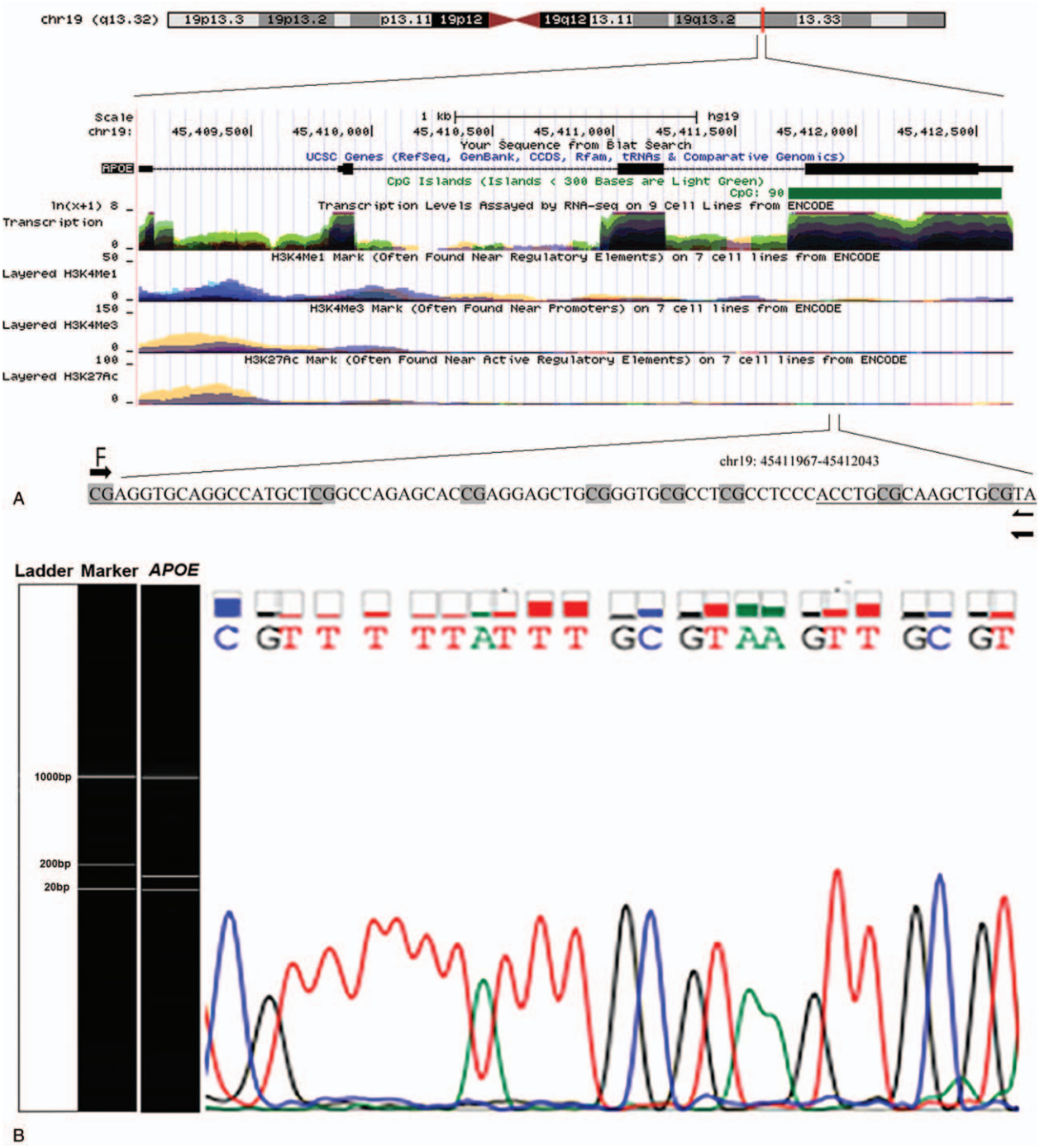

Figure 2. Target sequences on the CpG island regions of *APOE*.

Further breakdown analysis by ApoE protein types showed that the genotype frequency of *SIRT1* rs7895833 in the ApoE E4 was significantly different between the MCI group and the control group (Table 4, $P = .005$). And this suggested that the association of *SIRT1* rs7895833 with MCI was dependent on *APOE* ε4 status.

Meanwhile, our results did not find statistically significant association of the rest polymorphisms with MCI (Table 2, $P > .05$), and our results showed that the minor allele frequencies (MAFs) of the four tested polymorphisms were similar in 1000 Genomes samples and our control samples (Supplemental Table 2, http://links.lww.com/MD/D105). In this study, the methylation level of the *APOE* gene was also examined. Our results showed that the *APOE* methylation was significantly different between the MCI group and the control group (Table 5, $P = .021$), especially in males (Table 5, $P = .006$). Further subgroup analysis showed that *APOE* methylation levels were significantly associated with MCI in the non-*APOE* ε4 group (Table 5, $P = .009$), especially in males (Table 5, $P = .015$). In addition, there was no correlation of age and clinical indicators with *APOE* methylation (Supplemental Table 3, http://links.lww.com/MD/D105, $P > .05$).

4. Discussion

The *APOE* ε4 allele is highly associated with AD and is the strongest genetic risk factor for AD.[27] The ApoE E4 protein type containing the ε4 allele was considered to be a risk factor for AD, and the ApoE E3 and E2 protein types were a neutral factor and a protective factor for AD, respectively.[28] The relationship between *APOE* gene polymorphism and MCI is still controversial. Our results showed that *APOE* ε4 had no correlation with the incidence of MCI in Xinjiang Uygur population in China, and *APOE* hypermethylation level was found to be significantly correlated with the incidence of MCI, especially in the non-*APOE* ε4 male group. The results suggested that *APOE* hypermethylation level was not affected by *APOE* ε4 allele. Our study did not find clinical phenotypes such as blood lipids and blood glucose related to *APOE* methylation levels. Further research is needed to investigate the role of *APOE* methylation in the male MCI with non-*APOE* ε4 allele.

Damage to lipid balance in the body can seriously impair neuronal function and trigger neurodegenerative diseases.[29] Increased serum cholesterol is a known vascular risk factor for AD.[30,31] In some studies, the use of HMGCR inhibitors (statins) to treat hypercholesterolemia may provide a degree of neuroprotection in the advanced development of AD.[32–35] In addition, statin therapy reduces the phosphorylation-Tau protein content of cerebrospinal fluid.[36] Genome-wide study indicated that the *HMGCR* gene was one of the hotspot genes of AD,[37] and studies confirmed that *HMGCR* gene polymorphism was associated with AD.[38,39] A recent study also found *HMGCR* gene polymorphism was a risk factor for the conversion of MCI to AD.[40] Our study showed that *HMGCR* rs3846662 was associated with the onset of MCI in Xinjiang Uygur population in China, especially in females with non-*APOE* ε4 allele or ApoE E2 protein type. This result suggests that *HMGCR* rs3846662 may trigger MCI by affecting the body's lipid metabolism balance, which may have a more significant effect on women and has no synergistic relationship with *APOE* ε4.

SIRT1 protein has a neuroprotective effect,[8] and SIRT1 protein was shown to prevent the pathological accumulation of Aβ protein in the brain.[41] A significant increase in cerebrospinal fluid Aβ was found in patients with MCI,[42] and the level of SIRT1 in serum was significantly decreased in MCI.[10] Our results showed that the *SIRT1* rs7895833 were not significantly associated with MCI, but in the *APOE* ε4 and ApoE E4 subgroup analyses, *SIRT1* rs7895833 was found to be associated with

Table 2

Comparisons of genotype and allele frequencies between cases and controls.

SNPs	MCI; control (MM/Mm/mm)	*p* genotype	MCI; control (M/m)	*p* allele	OR (95%CI)
Total					
APHIB rs1047552 (T > G)	35/7/1; 95/28/2	0.593	77/9; 218/32	0.568	1.256 (0.573–2.750)
SIRT1 rs7895833 (A > G)	14/19/10; 31/66/28	0.549	47/39; 128/122	0.581	1.149 (0.703–1.878)
HMGCR rs3846662 (G > A)	19/20/4; 35/67/23	0.102	58/28; 137/113	0.040	1.709 (1.021–2.860)
PRNP rs1799990 (A > G)	27/15/1; 64/51/10	0.325	69/17; 179/71	0.116	1.610 (0.886–2.927)
Males					
APHIB rs1047552 (T > G)	18/4/0; 52/11/2	1.000	40/4; 115/15	0.785	1.304 (0.409–4.161)
SIRT1 rs7895833 (A > G)	9/7/6; 19/36/10	0.149	25/19; 74/56	0.990	0.996 (0.499–1.986)
HMGCR rs3846662 (G > A)	10/10/2; 23/34/8	0.691	30/14; 80/50	0.430	1.339 (0.648–2.768)
PRNP rs1799990 (A > G)	13/9/0; 36/26/3	0.914	35/9; 98/32	0.574	1.270 (0.551–2.924)
Females					
APHIB rs1047552 (T > G)	17/3/0; 43/17/0	0.233	37/3; 103/17	0.270	2.036 (0.564–7.348)
SIRT1 rs7895833 (A > G)	5/12/4; 12/30/18	0.623	22/20; 54/66	0.409	1.344 (0.665–2.719)
HMGCR rs3846662 (G > A)	9/10/2; 12/33/15	0.079	28/14; 57/63	0.032	2.211 (1.060–4.609)
PRNP rs1799990 (A > G)	14/6/1; 28/25/6	0.306	34/8; 81/37	0.128	1.941 (0.819–4.601)

Table 3

Distribution frequencies of genotypes and alleles of subgroup analyse based on *APOE* ε4 allele in MCI cases and controls.

SNPs	MCI; control (MM/Mm/mm)	*p* genotype	MCI; control (M/m)	*p* allele	OR (95%CI)
APOE ε4+					
APHIB rs1047552 (T > G)	7/1/0; 27/6/0	1.000	15/1; 60/6	1.000	1.500 (0.168–13.420)
SIRT1 rs7895833 (A > G)	5/1/2; 5/23/5	0.005	11/5; 33/33	0.177	2.200 (0.668–7.032)
HMGCR rs3846662 (G > A)	2/5/1; 10/17/6	1.000	9/7; 37/29	0.989	1.008 (0.335–3.030)
PRNP rs1799990 (A > G)	5/3/0; 18/13/2	1.000	13/3; 49/17	0.749	1.503 (0.382–5.924)
APOE ε4-					
APHIB rs1047552 (T > G)	28/6/1; 68/22/2	0.634	62/8; 158/26	0.572	1.275 (0.548–2.969)
SIRT1 rs7895833 (A > G)	9/18/8; 26/43/23	0.894	36/34; 95/89	0.977	0.992 (0.572–1.721)
HMGCR rs3846662 (G > A)	17/15/3; 25/50/17	0.057	49/21; 100/84	0.024	1.960 (1.089–3.528)
PRNP rs1799990 (A > G)	12/12/1; 46/38/8	0.785	56/14; 130/54	0.133	1.662 (0.854–3.234)
HMGCR rs3846662 (G > A)					
Males and *APOE* ε4-	8/8/1; 18/25/7	0.571	24/10; 61/39	0.316	1.534 (0.662–3.554)
Females and *APOE* ε4-	9/7/2; 7/25/10	0.036	25/11; 39/45	0.021	2.622 (1.145–6.006)

Table 4

Distribution frequencies of genotypes and alleles of subgroup analyse based on *APOE* gene protein phenotype in MCI cases and controls.

SNPs	MCI; control (MM/Mm/mm)	*p* genotype	MCI; control (M/m)	*p* allele	OR (95%CI)
ApoE E2					
APHIB rs1047552 (T > G)	4/1/0; 12/3/0	1.000	9/1; 27/3	1.000	1.000 (0.092–10.865)
SIRT1 rs7895833 (A > G)	1/4/0; 3/6/6	0.222	6/4; 12/18	0.300	2.250 (0.522–9.697)
HMGCR rs3846662 (G > A)	5/0/0; 4/8/3	0.021	10/0; 16/14	0.007	0.615 (0.454–0.834)
PRNP rs1799990 (A > G)	1/4/0; 6/7/1	0.702	6/4; 19/9	0.709	0.711 (0.160–3.163)
ApoE E3					
APHIB rs1047552 (T > G)	25/5/0; 60/20/2	0.445	55/5; 140/24	0.214	1.886 (0.685–5.192)
SIRT1 rs7895833 (A > G)	9/14/8; 25/39/18	0.910	32/30; 89/75	0.721	0.899 (0.501–1.614)
HMGCR rs3846662 (G > A)	13/15/3; 23/43/16	0.256	41/21; 89/75	0.108	1.645 (0.895–3.025)
PRNP rs1799990 (A > G)	22/8/1; 44/31/7	0.224	52/10; 119/45	0.077	1.966 (0.921–4.199)
ApoE E4					
APHIB rs1047552 (T > G)	6/1/0; 23/5/0	1.000	13/1; 51/5	1.000	1.275 (0.137–11.873)
SIRT1 rs7895833 (A > G)	4/1/2; 3/21/4	0.005	9/5; 27/29	0.282	1.933 (0.575–6.499)
HMGCR rs3846662 (G > A)	1/5/1; 8/16/8	0.636	7/7; 32/32	1.000	1.000 (0.315–3.179)
PRNP rs1799990 (A > G)	4/3/0; 14/13/1	1.000	11/3; 41/15	1.000	1.341 (0.329–5.478)
HMGCR rs3846662 (G > A)					
Males and ApoE E2	1/0/0; 2/3/1	1.000	2/0; 7/5	0.505	1.714 (1.063–2.765)
Females and ApoE E2	4/0/0; 2/5/2	0.063	8/0; 9/9	0.023	2.000 (1.260–3.174)

MCI. We hypothesized that the *SIRT1* rs7895833 polymorphism may synergize with *APOE* ε4, and thus increase the risk of MCI in the ApoE E4 carriers.

There are some limitations of our study that should be taken with caution. Firstly, the sample size in our study is moderate, and there will be a chance of class II errors in our results. Future study with expanded sample size should be performed to verify the results of this study. In addition, the subjects in this study were all elderly people who usually had other known or unknown diseases. Although we tried to control the confounding factors, there may have a potential to produce false positive results. In the future, independent research is needed to confirm our findings.

5. Conclusions

This study found that a *APOE*-ε4-independent association of *APOE* gene hypermethylation with MCI in male Chinese Uygur population. And our results indicated that *HMGCR* rs3846662 was significantly associated with female MCI independent of *APOE* ε4. In addition, we also found that *SIRT1* rs7895833 may interact with *APOE* ε4 to participate in the risk of Chinese Uygur MCI. Further study is need to validate our findings and hypothesis.

Table 5

The differences of *APOE* methylation between males and females.

	***APOE* methylation**			
	MCI	**Control**	***t***	***P***
Total	0.007 ± 0.003	0.006 ± 0.003	2.338	**.021**
Male	0.008 ± 0.003	0.006 ± 0.003	2.823	**.006**
Female	0.007 ± 0.003	0.007 ± 0.004	0.661	.51
APOE ε4 (+)	0.008 ± 0.004	0.006 ± 0.005	0.58	.565
Male	0.008 ± 0.005	0.005 ± 0.003	1.281	.217
Female	0.007 ± 0.005	0.007 ± 0.005	−0.078	.938
APOE ε4 (−)	0.007 ± 0.003	0.006 ± 0.003	2.64	**.009**
Male	0.008 ± 0.003	0.006 ± 0.003	2.496	**.015**
Female	0.007 ± 0.028	0.006 ± 0.003	1.151	.254

P values < 0.05 were in bold fonts.

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Author contributions

Conceptualization: Xiaohui Zhou, Qinwen Wang, Shiwei Duan.
Data curation: Ting Zou, Yali Duan, Xiuru Ying.
Formal analysis: Ting Zou, Xiuru Ying.
Investigation: Ting Zou, Yali Duan, Wei Chen, Meisheng Zhu, Abuliz Pari, Kader Alimu, Haijun Miao, Keyim Kabinur, Lei Zhang.
Methodology: Ting Zou, Yali Duan, Wei Chen, Guili Liu, Yongjie Zhao.
Project administration: Ting Zou.
Supervision: Xiaohui Zhou, Qinwen Wang, Shiwei Duan.
Validation: Ting Zou, Shiwei Duan.
Writing – original draft: Ting Zou.
Writing – review & editing: Ting Zou.
Ting Zou orcid: 0000-0002-6744-4648.

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