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

Abstract
Our study investigated the association of five genes with MCI in the Xinjiang Uyghur 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 e4 group (P = .024), especially in the females with non-APOE e4. 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 e4 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 e4 group (P = .009), especially in men (P = .015).

This study found a significant association of HMGCR rs3846662 with MCI in females independent of APOE e4. In contrast, we revealed that the association of SIRT1 rs7895833 with MCI was dependent on with APOE e4. We also showed that hypermethylation of APOE in MCI was independent of APOE e4.

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
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 e4 carriers are prone to Aβ deposition and Tau protein entanglement, which may increase the risk of MCI,[5,6] while APOE e2 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 Huifu 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) | χ² / 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 | 0.948 |
| Hypertension (yes/no) | 20/23 | 51/74             | 0.428 | 0.513 |
| Diabetes (yes/no) | 1/42         | 1/124             | 0.633 | 0.448 |
| Age (years)     | 71.56 ± 9.32  | 71.77 ± 8.16      | /     | 0.733 |
| Fasting blood glucose (FBG) (mmol/L) | 4.77 ± 1.44 | 4.63 ± 1.10 | / | 0.897 |
| Triglyceride (TG) (mmol/L) | 1.69 ± 1.01 | 1.83 ± 1.19 | / | 0.406 |
| Total cholesterol (TC) (mmol/L) | 4.98 ± 1.00 | 4.79 ± 1.20 | 0.098 | 0.365 |
| High density lipoprotein (HDL) (mmol/L) | 1.50 ± 0.32 | 1.49 ± 0.59 | / | 0.493 |
| Low density lipoprotein (LDL) (mmol/L) | 3.46 ± 0.88 | 3.14 ± 1.01 | 1.898 | 0.059 |
| Systolic blood pressure (SBP) (mm Hg) | 147.65 ± 29.20 | 140.52 ± 25.79 | 1.511 | 0.133 |
| Diastolic blood pressure (DBP) (mm Hg) | 79.93 ± 15.50 | 76.11 ± 15.91 | / | 0.143 |
| 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 ddH2O, 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 conversion 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 to 2.860), especially in women (Table 2, P = .032, OR = 2.211, 95% CI = 1.060 to 4.609). Further subgroup analysis by APOE e4 allele showed that HMGCR rs3846662 was statistically associated with MCI in the non-APOE e4 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 e4 (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 e4.

In addition, the distribution of SIRT1 rs7895833 genotype frequencies in the APOE e4 group was statistically significant between MCI group and control group (Table 3, P = .005).
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 e4 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 e4 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, \(P > .05\)).

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

The APOE e4 allele is highly associated with AD and is the strongest genetic risk factor for AD.[27] The ApoE E4 protein type containing the e4 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 e4 had no correlation with the incidence of MCI in Xinjiang Uygur population in China, and APOE hpermethylation level was found to be significantly correlated with the incidence of MCI, especially in the non-APOE e4 male group. The results suggested that APOE hypermethylation level was not affected by APOE e4 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 e4 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 e4 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 e4.

SIRT1 protein has a neuroprotective effect,[41] and SIRT1 protein was shown to prevent the pathological accumulation of Ab protein in the brain.[41] A significant increase in cerebrospinal fluid Ab 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 e4 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 (M/M/m/m) | p genotype | MCI; control (M/m) | p allele | OR (95%CI) |
|-------------------|------------------------|------------|--------------------|----------|------------|
| Total             |                        |            |                    |          |            |
| APOB rs1047552    | 35/7/1; 95/28/2        | 0.503      | 77/9; 218/32       | 0.568    | 1.256 (0.573–2.750) |
| SIRT1 rs7895833   | 14/19/10; 31/66/28     | 0.549      | 47/39; 128/122     | 0.581    | 1.149 (0.703–1.878) |
| HMGCR rs3846662   | 19/20/4; 35/67/23      | 0.102      | 58/28; 137/113     | 0.040    | 1.709 (1.021–2.860) |
| PRNP rs179990     | 27/15/1; 64/51/10      | 0.325      | 69/17; 179/71      | 0.116    | 1.610 (0.886–2.927) |
| Males             |                        |            |                    |          |            |
| APOB rs1047552    | 18/4/0; 52/11/2        | 1.000      | 40/4; 115/15       | 0.785    | 1.304 (0.409–4.161) |
| SIRT1 rs7895833   | 9/7/6; 19/36/10        | 0.149      | 25/19; 74/56       | 0.990    | 0.996 (0.499–1.986) |
| HMGCR rs3846662   | 10/10/2; 23/34/8       | 0.691      | 30/14; 89/50       | 0.430    | 1.339 (0.648–2.708) |
| PRNP rs179990     | 13/9/0; 36/26/3        | 0.914      | 35/9; 98/32        | 0.574    | 1.270 (0.551–2.924) |
| Females           |                        |            |                    |          |            |
| APOB rs1047552    | 17/3/0; 41/17/0        | 0.233      | 37/3; 103/17       | 0.270    | 2.036 (0.564–7.348) |
| SIRT1 rs7895833   | 5/12/4; 12/30/18       | 0.623      | 22/20; 54/66       | 0.409    | 1.344 (0.665–2.719) |
| HMGCR rs3846662   | 9/10/2; 12/33/15       | 0.079      | 28/14; 57/63       | 0.032    | 2.211 (1.080–4.609) |
| PRNP rs179990     | 14/6/1; 28/25/6        | 0.306      | 34/8; 81/37        | 0.128    | 1.941 (0.919–4.601) |

### Table 3

Distribution frequencies of genotypes and alleles of subgroup analyse based on APOE e4 allele in MCI cases and controls.

| SNPs              | MCI; control (M/M/m/m) | p genotype | MCI; control (M/m) | p allele | OR (95%CI) |
|-------------------|------------------------|------------|--------------------|----------|------------|
| APOE e4+          |                        |            |                    |          |            |
| APOB rs1047552    | 7/1/0; 27/6/0          | 1.000      | 15/1; 60/6         | 1.000    | 1.500 (0.168–13.420) |
| SIRT1 rs7895833   | 5/1/2; 52/3/5          | 0.005      | 11/5; 33/33        | 0.177    | 2.200 (0.668–7.032) |
| HMGCR rs3846662   | 25/5/1; 10/17/6        | 1.000      | 9/7; 37/29         | 0.989    | 1.008 (0.335–3.030) |
| PRNP rs179990     | 5/3/0; 18/13/2         | 1.000      | 13/3; 49/17        | 0.749    | 1.503 (0.382–5.924) |
| APOE e4-          |                        |            |                    |          |            |
| APOB rs1047552    | 28/6/1; 68/22/2        | 0.634      | 62/8; 158/26       | 0.572    | 1.275 (0.548–2.969) |
| SIRT1 rs7895833   | 9/16/5; 24/43/23       | 0.949      | 36/54; 95/89       | 0.972    | 0.992 (0.572–1.721) |
| HMGCR rs3846662   | 17/15/3; 26/50/17      | 0.057      | 49/21; 100/44      | 0.024    | 1.960 (0.689–5.290) |
| PRNP rs179990     | 12/12/1; 46/38/8       | 0.785      | 56/14; 130/54      | 0.133    | 1.662 (0.854–3.234) |
| HMGCR rs3846662   | 12/9/1; 46/38/8        | 0.785      | 56/14; 130/54      | 0.133    | 1.662 (0.854–3.234) |
| Males and APOE e4+| 8/8/1; 18/25/7         | 0.571      | 24/10; 61/39       | 0.316    | 1.534 (0.662–3.554) |
| Females and APOE e4-| 9/7/2; 7/25/10       | 0.036      | 25/11; 39/45       | 0.021    | 2.622 (1.145–6.006) |
HMGCR rs3846662 (G > A) was significantly associated with female MCI independent of APOE e4. We hypothesized that the SIRT1 rs7895833 polymorphism may synergize with APOE e4, 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-e4-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 e4. In addition, we also found that SIRT1 rs7895833 may interact with APOE e4 to participate in the risk of Chinese Uygur MCI. Further study is need to validate our findings and hypothesis.

Acknowledgments

We are grateful to staff who joined in the epidemiological survey in 2015 in Hetian Region, Xinjiang province, China. This research was supported by the grants from the National Natural Science Foundation of China (No. 81360064), the High Technology Research and Development Projects of Xinjiang Province (No. 201517104), National key research and development program (2016YFC1305900), and K. C. Wong Magna Fund in Ningbo University.

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 Kabnur, 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.

Table 4

| SNPs       | Control (MM/Mm/mm) | MCI (MM/Mm/mm) | p genotype | MCI (Mm/m/m) | p allele | OR (95%CI) |
|------------|--------------------|----------------|-------------|--------------|----------|-----------|
| ApoE E2    |                    |                |             |              |          |           |
| APOB rs1047552 (T > G) | 4/10; 12/30       | 1.000          | 9/1; 27/3   | 1.000        | 1.000    | 0.992–10.865 |
| SIRT1 rs7895833 (A > G) | 1/4; 0/3/6        | 0.222          | 6/4; 12/18  | 0.300        | 2.250    | 0.522–9.697  |
| HMGCR rs3846662 (G > A) | 5/0; 0/4/8        | 0.021          | 10/0; 16/14 | 0.007        | 0.615    | 0.454–0.834  |
| PRNP rs1799990 (A > G) | 1/4; 0/6/7        | 0.702          | 6/4; 19/9   | 0.709        | 0.711    | 0.160–3.163  |
| ApoE E3    |                    |                |             |              |          |           |
| APOB 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  | 0.010          | 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; 69/75| 0.108        | 1.645    | 0.895–3.025  |
| PRNP rs1799990 (A > G) | 22/21/1; 44/31/7  | 0.224          | 52/10; 119/45| 0.077        | 1.966    | 0.021–4.190  |
| ApoE E4    |                    |                |             |              |          |           |
| APOB rs1047552 (T > G) | 6/10; 23/5/0      | 1.000          | 13/1; 51/5  | 1.000        | 1.275    | 0.137–11.873 |
| SIRT1 rs7895833 (A > G) | 4/1/2; 3/2/14     | 0.005          | 9/5; 27/29  | 0.282        | 1.933    | 0.576–6.499  |
| HMGCR rs3846662 (G > A) | 1/5/1; 8/16/8     | 0.036          | 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) | 2/2/0; 8/7/1      | 0.683          | 8/0; 7/9   | 0.023        | 2.000    | 1.260–3.174  |

Table 5

| APOE methylation | MCI (MM/Mm/mm) | Control (MM/Mm/mm) | p genotype | OR (95%CI) |
|------------------|---------------|-------------------|-------------|-----------|
| Total            | 0.007 ± 0.003 | 0.006 ± 0.003     | 2.338       | 0.021     |
| Male             | 0.006 ± 0.003 | 0.005 ± 0.003     | 2.832       | 0.006     |
| Female           | 0.007 ± 0.003 | 0.007 ± 0.004     | 1.661       | 0.5       |
| APOE e4 (+)      | 0.006 ± 0.004 | 0.006 ± 0.005     | 0.58        | 0.565     |
| Male             | 0.008 ± 0.005 | 0.005 ± 0.003     | 1.281       | 0.217     |
| Female           | 0.007 ± 0.005 | 0.007 ± 0.005     | -0.078      | 0.938     |
| APOE e4 (-)      | 0.007 ± 0.003 | 0.006 ± 0.003     | 2.64        | 0.009     |
| Male             | 0.008 ± 0.003 | 0.006 ± 0.003     | 2.496       | 0.015     |
| Female           | 0.007 ± 0.028 | 0.006 ± 0.003     | 1.151       | 0.254     |

P values < 0.05 were in bold fonts.

References

[1] Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med 2004;255:183–94.
[2] Winblad B, Palmer K, Kivipelto M, et al. Mild cognitive impairment—beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med 2004;255:240–6.
[3] Petersen RC, Roberts RO, Knopman DS, et al. Mild cognitive impairment: ten years later. Arch Neurol 2009;66:1151–7.
[4] Farias ST, Mungas D, Reed BR, et al. Progression of mild cognitive impairment to dementia in clinic- vs community-based cohorts. Arch Neurol 2009;66:1151–7.
[5] Jowkar B, Kojuri J, Kohoulat N, et al. Academic resilience in education: the role of achievement goal orientations. J Adv Med Educ Prof 2014;2:33–8.
[6] Brainard CJ, Reyna VF, Petersen RC, et al. The apolipoprotein E genotype predicts longitudinal transitions to mild cognitive impairment but not to Alzheimer’s dementia: findings from a nationally representative study. Neuropsychology 2013;27:86–94.

[7] Oveisgharan S, Buchman AS, Yu L, et al. APOE epsilon2epsilon4 genotype, incident AD and MCI, cognitive decline, and AD pathology in older adults. Neurology 2018;90:e2127–34.

[8] Qin W, Yang T, Ho L, et al. Neuronal SIRT1 activation as a novel mechanism underlying the prevention of Alzheimer disease amyloid neuropathology by calorie restriction. J Biol Chem 2006;281:21745–54.

[9] Lu J, Li C, Li X, et al. Cholesterol and tau protein—findings in Alzheimer’s and Niemann Pick C’s disease. Pharmacop-sychiatry 2003;36(Suppl 2):S120–126.

[10] Kumar R, Chaterjee P, Sharma PK, et al. Sirtuin1: a promising serum protein marker for early detection of Alzheimer’s disease. PLoS One 2013;8:e61560.

[11] Watanabe N, Tomita T, Sato C, et al. Pen-2 is incorporated into the gamma-secretase complex through binding to transmembrane domain 4 of presenilin 1. J Biol Chem 2005;280:41967–75.

[12] Tallach RE, Ball DR. Routine pre-oxygenation. Anaesthesia 1997;52:557–61.

[13] Lee SF, Shah S, Yu C, et al. A conserved GXXXG motif in APH-1 is critical for assembly and activity of the gamma-secretase complex. J Biol Chem 2004;279:4144–52.

[14] Lee SF, Shah S, Yu C, et al. A conserved GXXXG motif in APH-1 is critical for assembly and activity of the gamma-secretase complex. J Biol Chem 2004;279:4144–52.

[15] Shirotani K, Edbauer D, Prokop S, et al. Identification of distinct gamma-secretase complexes with different APH-1 variants. J Biol Chem 2004;279:41340–5.

[16] Bekris LM, Tsuang DW, Peskind ER, et al. Cerebrospinal fluid Abeta42 levels and APP processing pathway genes in Parkinson’s disease. Mov Disord 2013;30:936–44.

[17] Li B. The pathogenesis of soluble Pr fragments containing Abeta binding sites. Virus Res 2016;211:194–8.

[18] Boundy CR, de Souza C, et al. Polymorphism of the prion protein is associated with cognitive impairment in the elderly: the EVA study. Neurology 1998;51:734–7.

[19] Koudinov AR, Berezov TT, Koudinova NV. Alzheimer’s amyloid beta and lipid metabolism: a missing link? FASEB J 1998;12:1097–9.

[20] Shankar KL, Li H, et al. Effect of statins on Alzheimer’s disease biomarkers in cerebrospinal fluid. J Alzheimers Dis 2006;10:399–406.

[21] Blacker D, Bertram L, Saunders AJ, et al. Results of a high-resolution genome screen of 437 Alzheimer’s disease families. Hum Mol Genet 2003;12:23–32.

[22] Rockwood K, Kirkland S, Hogan DB, et al. Use of lipid-lowering agents, indication bias, and the risk of dementia in community-dwelling elderly people. Arch Neurol 2002;59:223–7.

[23] Jack H, Zornberg GL, Jick SS, et al. Statins and the risk of dementia. Lancet 2000;356:1627–31.

[24] rocker RG, Li G, Petric E, et al. Effect of statins on Alzheimer’s disease risk and prevalence of dementia and Alzheimer disease associated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Arch Neurol 2000;57:1439–43.