The Association between Apolipoprotein E Gene Polymorphism and Mild Cognitive Impairment among Different Ethnic Minority Groups in China

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1. Introduction

Apolipoprotein E (apoE) is a plasma protein involved in regulating the body’s metabolism of lipoproteins and cholesterol balance. It has an important role in the neurobiological system [1]. Human apoE has three isoforms, Ε3, Ε4, and Ε2, due to the cysteine-arginine interchanges at codons 112 and 158 [2]. The most frequently occurring allele is Ε3, followed by Ε4 and Ε2 [1].

The apoE Ε4 allele has been widely studied as a risk factor for mild cognitive impairment (MCI) and Alzheimer’s disease (AD), but its impact has been found to differ across ethnic groups [3–7]. See et al. reviewing the frequency of the apoE alleles in different ethnic groups found that the relative frequency of Ε4 was the lowest in the Chinese sample [8]. This raised a question as to whether the Chinese people did in fact benefit from the reduced frequency of the apoE Ε4 allele.

The prevalence of MCI was estimated to be over 18.5% among Chinese people aged 55 years or older [9], with about seven million Chinese people suffering from AD among people aged 55 years or older [10].

Within the Chinese population, studies have shown that apoE Ε4 increases the risk of MCI in the elderly Han ethnic group (the majority group) [11, 12], but few studies have examined the association between the Ε4 allele and MCI among different ethnic minority groups in China, although the frequency of apoE genotypes has been found to differ across different ethnic groups [13–15]. China has fifty-five national minorities, among which the Hui ethnic group is the largest minority group. The Chinese Hui ethnic group has descended from Arab and Persian Muslim immigrants [16]. They share the same language, customs, and living environments with the local Han majority but are different in terms of genetic background [17]. Deng et al. investigated...
the diversity distributions of 15 short tandem repeats (STRs) loci in a sample of the Hui ethnic group comparing them with other Chinese ethnic groups. The results showed a significant difference between the Hui and other ethnic groups in some loci [18]. In addition, previous studies of community residents showed that the Hui ethnic group had a higher prevalence of abnormal lipid metabolism than the local Han population [19]. However, there is no study focused on the frequency of the apoE e4 allele and the association between the e4 allele and MCI in the Hui ethnic minority.

In the current study, we aim to compare the relative frequency of the apoE alleles e2, e3, and e4 in a community-based sample of the Hui ethnic group with a comparable sample of the Han ethnic group in Mainland China and to examine the association between apoE gene polymorphism and MCI in those two groups.

2. Materials and Methods

2.1. Subjects. Five communities were selected from two cities using a convenience cluster sampling method. Among them, three communities were from Yinchuan (the biggest city in the Ningxia province, and over 80% of the population is Han ethnicity) and two communities were from Wu Zhong (the second biggest city in the Ningxia province, and over 80% of the population is Hui ethnicity). Individuals aged 55 years or older who had permanent residency and agreed to participate were included in the study. Those who could not complete the survey due to vision and hearing disabilities, a long history of alcohol consumption, or serious diseases were excluded. Demographic information was collected using a questionnaire designed by the research team, and the ethnic identity was verified by residency registration information (called hukou in Chinese) authorized by the local government. The residency registration system is the basic institution which documents population information and distributes public resources in China [20].

Of 1022 participants enrolled in the study, 924 (90.5%) completed the entire examination, including 306 Hui ethnic people (mean age is 65.4 years; standard deviation (SD) is 6.8) and 618 Han ethnic people (mean age is 66.9 years; SD is 6.5). This study was approved by the Institutional Review Board of the Ningxia Medical University. Participants provided written informed consent prior to the survey.

2.2. Neuropsychological Testing and Physical Examination. All the participants had a face-to-face interview performed by trained medical students using a structured questionnaire, which included questions on smoking, alcohol use, memory, and medical history. A series of tests including the Minimental State Exam (MMSE) [21], Activities of Daily Living Scale (ADL) [22], and the Geriatric Depression Scale (GDS) [23] were administered by two clinicians from the Geriatrics Department of Ningxia Medical University. MCI was diagnosed according to Peterson's criteria [24]. One hundred and eighty-one people (19.6%) met the criteria for MCI, including 66 Hui and 115 Han ethnic people (Table 1). All participants underwent a careful physical examination in community health care centers to obtain the blood pressure and disease history.

2.3. ApoE Gene Polymorphism Test. Genomic DNA was isolated from venous blood leukocytes using a genomic DNA extraction and purification kit following the manufacturer's protocol (Wizard, Genomic DNA Purification Kit; Promega Company, USA). The primer was made by Invitrogen Company and diluted and kept at −20°C. An 89 bp PCR product encompassing acid position rs429835 (112) was produced by using the forward primer 5' - CCGGCACGCTGCTCAACG-3' and the reverse primer 5' - CGGTACTGACCACCGGCGC-3'. A 70 bp PCR product encompassing acid position rs7412 (158) was produced by using the forward primer 5' - GCTGCATCAGCGCTCCTCGC-3' and the reverse primer 5' - GGCCCCGGCCTGATCAT-3'.

ApoE gene polymorphism was detected by the high resolution melting (HRM) curve method [25]. Instruments were undertaken in a Roche Light Cycler 480 (Roche, USA). The reaction system was composed of 5 µL reaction mixture, 0.5 µL primer mix, 0.5 µL fluorescein EVA, and 3 µL pure water. The total volume was 9 µL. The system was placed in a 1.5 mL Eppendorf tube and then in a vortex momentarily for mixing. The reaction system was added to Roche 96 pore plates, and each pore plate was added 1 µL DNA moldboard. A computerized test was run after shocking. All detections were completed by professional technicians at the Biochip Ningxia Center following the manufacturer's instructions. In order to control for an accurate genotype call, twelve DNA samples were retested using the dideoxy-mediated chain-termination method in an independent Lab (Genex, China). All the genotypes were detected by the sequencing method consistent with that detected by the HRM.

2.4. Statistical Analyses. We identified the carriers of the e2 allele, including genotypes e2e2 and e2e3, and the carriers of the e4 allele, including genotypes e3e4 and e4e4, and e3e3 as control. A gene risk index model, widely used in other studies, was used to evaluate the variation in apoE polymorphisms [26]. The model assigned +1, 0, or −1 for e2, e3, or e4 allele, respectively. The genotypes e2e2, e2e3, e2e4, e3e3, e3e4, and e4e4 then had scores of +2, +1, 0, 0, −1, and −2, respectively. The distributions of apoE genotypes, the frequency of the alleles (e2, e3, and e4), e2 allele carrier, e4 allele carrier, and demographical variables between Han and Hui or between MCI and no MCI were analyzed using the R × C chi-square test. Age and apoE risk index were analyzed using Student's t-test. Logistic regression model was performed to obtain the odds ratios (OR) and 95% confidence intervals (CI) for the e2 or e4 allele carriers after controlling for age, gender, and level of education. Also, two separated logistic regression models performed stratified by ethnic. The statistically significant level was set at P < 0.05. All analyses were performed using the statistical package for social sciences version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Frequency of ApoE Allele and Genotype Distributions in the Hui and Han Ethnic Groups. There was no statistically significant difference in the distribution of the six kinds
Table 1: Characteristics of the studied population.

|                          | Total (N = 924) | Hui (N = 306) | Han (N = 618) | P value* |
|--------------------------|-----------------|---------------|---------------|---------|
| Age, mean (SD), and years| 66.4 (6.6)      | 65.4 (6.8)    | 66.9 (6.5)    | 0.001   |
| Gender, female, n (%)    | 571 (61.8)      | 183 (59.8)    | 388 (62.7)    | 0.380   |
| Education, n (%)         |                 |               |               |         |
| No formal education      | 239 (25.9)      | 106 (34.6)    | 133 (21.5)    |         |
| Primary school           | 253 (27.4)      | 89 (29.1)     | 164 (26.5)    | <0.001  |
| Junior high school       | 254 (27.5)      | 69 (22.5)     | 185 (29.9)    |         |
| Senior high school or above | 178 (19.3)    | 42 (13.7)     | 136 (22.0)    |         |
| Living arrangement, alone, n (%) | 187 (20.2) | 48 (15.7) | 139 (22.5) | 0.015 |
| Smoking, yes, n (%)      | 218 (23.6)      | 48 (15.6)     | 170 (27.5)    | <0.001  |
| Alcohol use, yes, n (%)  | 115 (12.4)      | 14 (4.5)      | 101 (16.3)    | <0.001  |
| TC mean (SD) (mmol/L)    | 4.7 (0.9)       | 4.6 (1.0)     | 4.8 (0.9)     | 0.051   |
| TG mean (SD) (mmol/L)    | 1.8 (0.8)       | 1.7 (0.9)     | 1.8 (0.8)     | 0.273   |
| HDL mean (SD) (mmol/L)   | 4.0 (1.0)       | 4.0 (1.0)     | 4.0 (1.0)     | 0.738   |
| LDL mean (SD) (mmol/L)   | 3.2 (0.6)       | 3.2 (0.7)     | 3.2 (0.6)     | 0.307   |
| SBP mean (SD) (mmHg)     | 149.7 (20.4)    | 151.2 (20.9)  | 148.9 (20.2)  | 0.104   |
| DBP mean (SD) (mmHg)     | 87.0 (11.5)     | 88.5 (11.4)   | 86.3 (11.4)   | 0.005   |
| MCI, yes, n (%)          | 181 (19.6)      | 66 (21.5)     | 115 (18.6)    | 0.286   |
| apoE genotype n (%)      |                 |               |               |         |
| ε2/2                     | 19 (2.1)        | 8 (2.6)       | 11 (1.8)      |         |
| ε2/3                     | 122 (13.2)      | 39 (12.4)     | 83 (13.4)     |         |
| ε2/4                     | 3 (0.3)         | 2 (0.7)       | 1 (0.2)       | 0.517   |
| ε3/3                     | 715 (77.4)      | 237 (77.2)    | 479 (77.5)    |         |
| ε3/4                     | 58 (6.3)        | 17 (5.5)      | 41 (6.6)      |         |
| ε4/4                     | 7 (0.8)         | 4 (1.3)       | 3 (0.5)       |         |
| apoE allele frequency, n (%) |             |               |               |         |
| ε2                       | 163 (8.8)       | 57 (9.3)      | 106 (8.6)     |         |
| ε3                       | 1,612 (87.1)    | 530 (86.3)    | 1082 (87.5)   | 0.752   |
| ε4                       | 75 (4.0)        | 27 (4.4)      | 48 (3.9)      |         |
| ε4 carrier, yes, n (%)   | 68 (7.4)        | 23 (7.5)      | 45 (7.2)      | 0.898   |
| ε2 carrier, yes, n (%)   | 144 (15.6)      | 49 (16.0)     | 95 (15.4)     | 0.800   |
| Risk index, mean (SD)    | 0.1 (0.5)       | 0.1 (0.5)     | 0.1 (0.5)     | 0.913   |

*P value of Hui with Han; TC: total cholesterol; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure.

of genotypes and three kinds of allele between the Hui and Han ethnic groups (Table 1). Compared with the Han ethnic group, the Hui ethnic group had higher levels of serum total cholesterol and diastolic blood pressure and lower proportions of smoking, alcohol use, and living alone and lower education level.

3.2. The Bivariate Association between ApoE ε4 Allele and MCI. Table 2 shows the association between apoE ε4 allele and MCI; we investigated the association between the apoE ε4 allele and MCI in the Hui and Han ethnic groups combined and separately. Consistent correlations between apoE ε4 allele and MCI were found in three subgroups. The proportion with the ε4 allele (7.5% versus 3.2%) and of ε4 carriers (14.4% versus 5.7) was significantly higher in the participants with MCI than those without MCI; P < 0.001. There was a significantly lower risk index score for those with MCI in the combined group (P = 0.002) and for the Han ethnic group (P = 0.007), but not in the Hui ethnic group (P = 0.118).

3.3. Logistic Regression Analysis for ApoE ε4 Allele and MCI (Table 3). After controlling for age, gender, and education level, the ε4 carrier was a risk factor for MCI in both the Hui (OR = 2.61) and the Han (OR = 2.36) ethnic groups. The ε2 carrier was protective for MCI in the Han ethnic group (OR = 0.48), but not in the Hui ethnic group.

4. Discussion

The present study found that there was no significant difference in apoE genotype and ε4 allele frequency between older Hui and Han ethnic groups. This finding is similar to that for the Chinese Uyghur ethnic people [15]. However, compared with the Saudi Arabia Muslims who are believed to be the
Table 2: Bivariate association between apoE ε4 allele and MCI.

| Allele frequency, n (%) | NO (N = 743) | MCI | Yes (N = 181) | P value |
|-------------------------|--------------|-----|--------------|---------|
| ε2 (n = 139)            |              |     |              | <0.001  |
| ε3 (n = 1299)           |              |     |              |         |
| ε4 (n = 48)             |              |     |              |         |
| ε2 carrier, yes, n (%)  |              |     |              |         |
| ε4 carrier, yes, n (%)  |              |     |              |         |
| Risk index, mean (SD)   |              |     |              |         |

Hui (n = 306)

| Allele frequency (%) | NO (N = 240) | MCI | Yes (N = 66) | P value |
|----------------------|--------------|-----|--------------|---------|
| ε2 (n = 46)          |              |     |              |         |
| ε3 (n = 418)         |              |     |              | <0.001  |
| ε4 (n = 16)          |              |     |              |         |
| ε2 carrier, yes, n (%) |              |     |              |         |
| ε4 carrier, yes, n (%) |              |     |              |         |
| Risk index, mean (SD) |              |     |              |         |

Han (n = 618)

| Allele frequency (%) | NO (N = 503) | MCI | Yes (N = 115) | P value |
|----------------------|--------------|-----|--------------|---------|
| ε2 (n = 93)          |              |     |              |         |
| ε3 (n = 881)         |              |     |              | <0.001  |
| ε4 (n = 32)          |              |     |              |         |
| ε2 carrier, yes, n (%) |              |     |              |         |
| ε4 carrier, yes, n (%) |              |     |              |         |
| Risk index, mean (SD) |              |     |              |         |

Table 3: Logistic regression of apoE gene polymorphism and MCI.

|                           | β   | SE  | Wald χ² | P     | OR   | 95% CI         |
|---------------------------|-----|-----|---------|-------|------|----------------|
| Total (n = 924)           |     |     |         |       |      |                |
| ethnicity                 | −0.33| 0.18| 3.39    | 0.066 | 0.71 | 0.49–1.02      |
| Risk index                | −0.46| 0.16| 7.80    | 0.005 | 0.63 | 0.45–0.87      |
| ε2 carrier                | −0.38| 0.27| 1.99    | 0.158 | 0.68 | 0.39–1.16      |
| ε4 carrier                | 0.89 | 0.28| 9.93    | 0.002 | 2.45 | 1.40–4.29      |
| Hui (n = 306)             |     |     |         |       |      |                |
| Risk index                | −0.53| 0.26| 0.74    | 0.389 | 0.79 | 0.47–1.33      |
| ε2 carrier                | 0.10 | 0.41| 0.06    | 0.807 | 1.10 | 0.49–2.47      |
| ε4 carrier                | 0.96 | 0.47| 4.04    | 0.044 | 2.61 | 1.02–6.66      |
| Han (n = 618)             |     |     |         |       |      |                |
| Risk index                | −0.53| 0.21| 6.36    | 0.012 | 0.58 | 0.38–0.88      |
| ε2 carrier                | −0.71| 0.34| 4.20    | 0.040 | 0.48 | 0.24–0.96      |
| ε4 carrier                | 0.86 | 0.34| 6.16    | 0.013 | 2.36 | 1.19–4.67      

Ancestors of the Chinese Hui ethnic people, the frequency of ε4 allele in the Hui ethnic group (3.9%) was close to that in the Arabs (3.9–6.3%) [27, 28]. The frequency of the ε4 allele is 4.4% in Hui and 3.9% in Han in current study; this finding agrees with previous studies which have reported that the frequency of apoE genotypes is significantly different in different races and ethnicities. The gene frequency of ε4 allele was from 12.4% to 19.8% in Caucasians, while the Chinese Han ethnic population has been found to have a lower frequency of the ε4 allele, ranging from 7.5% to 8.7% [8, 13, 26]. Regarding other Chinese minorities, the frequency of ε4 allele ranged from 4.9% in the Zhuang ethnic group [29] to 15.4% in the Mongolian ethnic group [30]. Studies have shown that apoE ε4 carrier increases the risk of MCI in the elderly Chinese Han ethnic group [11, 12]. However, except for the influence of gender, age, and level of education, current study found a consistent association
between the ε4 allele and MCI in both the Han and the Hui ethnic groups, and these findings were similar to that of other studies [31]. MCI was the most commonly accepted prodromal AD stage; this suggests that the apoE ε4 allele has relevance for MCI screening [32,33].

In contrast, apoE ε2 allele has been reported to be a protective factor for cognitive function in elderly people. Presence of the apoE ε2 allele was reported to have an association with improvement in episodic memory over time [34] and to reduce the risk of cognitive decline among older adults [35]. A recent study showed no significant association between apoE ε2 presence and MCI in Chinese Han ethnic people, but apoE ε2 was inversely associated with AD [36]. The current study found that the ε2 carrier was a protective factor for MCI in the Han ethnic group, but not in the Hui ethnic group. This difference may be due to the relatively smaller sample size for Hui participants and the relatively lower frequency of ε2 in Chinese population which reduced statistical power [26]. For the present study, the power for logistic model was 0.85 in Han groups and 0.73 in Hui groups, respectively.

5. Conclusions

In summary, this study has shown that apoE ε4 was a risk factor for MCI in both of the Han and Hui ethnic groups in China. But the association of apoE ε2 with MCI may differ in different ethnic groups in China. Further studies are needed to explore this effect among different populations.

6. Limitations

There are several limitations to the present study. First, the cross-sectional design of this study could only detect the association between genes and MCI, but not a causal relationship. Second, the samples were not randomly selected so that they could not represent the general population. In addition, the sample size of the Hui ethnic group was relatively smaller than that of the Han ethnic group, which might reduce the comparability of the two samples.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

Authors’ Contribution

ZhiZhong Wang and Wanrui Ma contributed equally to this work. Lan Liu and ZhiZhong Wang contributed to the study design, data analysis, and drafting of this paper. Wanrui Ma was involved in data preparation, data collection, and data analysis. Ye Rong was involved in data analysis and drafting of the paper.

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