Associations between apolipoprotein E gene polymorphisms and Alzheimer's disease risk in a large Chinese Han population

Ping Wu^{1,2}
 Hong-Lei Li¹
 Zhi-Jun Liu¹
 Qing-Qing Tao¹
 Miao Xu¹
 Qi-Hao Guo¹
 Zhen Hong¹
 Yi-Min Sun¹

¹Department of Neurology and Institute of Neurology, ²PET Center, Department of Nuclear Medicine, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, People's Republic of China

Objective: Apolipoprotein E gene (*APOE*) polymorphisms contributing to the risk of sporadic Alzheimer's disease (AD) have been identified for decades, but it has not been investigated in large AD samples of Chinese Han population.

Methods: We performed a cross-sectional study to explore the effect of *APOE* polymorphisms on sporadic AD in 875 sporadic AD patients and 1,195 cognitive normal controls of Chinese Han. Genotyping of *APOE* was determined by multiplex amplification refractory mutation system polymerase chain reaction.

Results: *APOE* $\epsilon 3\epsilon 4$ and $\epsilon 4\epsilon 4$ genotypes increased AD risk with dosage effect. The odds ratio (OR) of $\epsilon 3\epsilon 4$ was 1.89 and the OR of $\epsilon 4\epsilon 4$ was 15.64 compared with that of $\epsilon 3\epsilon 3$ in all the subjects. $\epsilon 2\epsilon 3$ genotype decreased AD risk in all the subjects (OR=0.64), female subgroup (OR=0.57), and late-onset AD subgroup (OR=0.60). However, neither $\epsilon 2\epsilon 2$ nor $\epsilon 2\epsilon 4$ affected AD risk. About the age at onset (AAO), the influence of *APOE* $\epsilon 4$ was only exhibited in late-onset AD subgroup, with 1 year lower in $\epsilon 4$ -positive ones than negative ones. Further analysis did not show the dosage effect of $\epsilon 4$ pertinent to AAO, though the AAO of $\epsilon 4\epsilon 4$ patients decreased by 2 years. $\epsilon 2$ did not affect the AAO of AD.

Conclusion: *APOE* $\epsilon 4$ is a strong risk factor of AD risk in Chinese Han population, and *APOE* $\epsilon 4\epsilon 4$ genotype might be related to the AAO of late-onset AD.

Keywords: sporadic, cross sectional study, dosage effect, age at onset

Introduction

Alzheimer's disease (AD) is the most common cause of senile dementia characterized by progressive decline in cognition and behaviors. The cause of AD was complex, and genetic factors contributed to its risk. Mutations on three genes of amyloid precursor protein, presenilin 1, and presenilin 2 are associated with rare familial early-onset AD (EOAD). As for the majority of sporadic AD (SAD), apolipoprotein E gene (*APOE*) was the only one confirmed to be related with SAD risks since 1993,^{1,2} and the results were replicated by many candidate genetic studies in different populations and different regions all around the world.³ Recent genome-wide association studies found that *APOE* is far more significantly related to AD risk than all the other candidate loci.^{4,5}

The *APOE* gene located in 19q13.2, encoding apoE, which consists of 299 amino acids, is a cholesterol carrier involved in lipid transportation and injury repair in the brain. *APOE* has three common isoforms termed $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, which could be determined by cysteine–arginine substitutions at residues 112 and 158. The frequencies of these three alleles are different among ethnics.⁶ Generally, $\epsilon 3$ is the most common allele, accounting for 60%–90% of the allelic variation. The

Correspondence: Yi-Min Sun
 Department of Neurology and Institute of Neurology, Huashan Hospital, Shanghai Medical College, Fudan University, 12 Wulumuqi Zhong Road, Shanghai 200040, People's Republic of China
 Tel/Fax +86 21 6248 3421
 Email ys2504@sina.com

$\epsilon 2$ constitutes 0%–20% of allelic variation and $\epsilon 4$ constitutes 10%–20% (http://asia.ensembl.org/Homo_sapiens/Variation/Population). The $\epsilon 4$ was proved to increase AD risk in a dose-dependent pattern and lower the age at disease onset compared with $\epsilon 4$ noncarriers.⁷ It was also reported as a risk factor for the conversion of mild cognitive impairment to AD.⁸ In contrast, $\epsilon 2$ was reported to have a “protective” effect on AD risk and to slower cognitive function decline than $\epsilon 2$ -negative status.⁹

The association between *APOE* polymorphisms and AD risk has been investigated in Caucasian, Hispanic, African American, Japanese, and small numbers of Chinese Han populations.^{10–12} Other studies of candidate genes and AD risk in Chinese Han population used *APOE* $\epsilon 4$ -carrying status as a stratification sign but did not focus on *APOE* itself.¹³ Here we investigated a large number of 875 SAD and 1,195 controls of Chinese Han to explore the association between *APOE* polymorphisms and AD risk.

Materials and methods

Subjects

A total of 875 SAD patients and 1,195 unrelated healthy controls were included in this cross-sectional study. All the subjects were from Chinese Han population. SAD patients were recruited from memory disorders clinics in Huashan Hospital between March 2007 and September 2013 with a median age of 72 years (range 48–100 years). Cognitively normal controls with age, sex, and origins similar to SAD patients were recruited from the community epidemiologic investigations (median age of 69 years, range 48–94 years). The enrollment procedure and the inclusion and exclusion criteria for SAD cases and controls were as previously reported.¹⁴ The diagnosis of AD was according to the criteria of Diagnostic and Statistical Manual of Mental Disorders IV revised. Written consents were obtained from subjects or their legally authorized caregivers. This study was approved by the ethics committee of Huashan Hospital.

Genotyping of *APOE*

Genomic DNA was extracted from peripheral blood using a Blood Genomic DNA Extraction Kit (TIANGEN, Shanghai, People's Republic of China). The *APOE* genotypes were determined by multiplex amplification refractory mutation system polymerase chain reaction according to the method previously described.¹⁵

Statistical analysis

Hardy–Weinberg equilibrium tests of *APOE* polymorphisms within the groups were performed using χ^2 analysis. The χ^2 test or Student's *t*-test was used to test for the differences between AD and control subjects in the distribution of sex, age at onset (AAO), and mini-mental state examination scores. The χ^2 test was used to compare the genotypes and allele frequencies between AD patients and control subjects. Odds ratio (OR) and the 95% confidence interval (CI) for testing possible associations between AD and control groups were determined by binary logistic regression analyses; AAO and sex were used as covariates. The potential effects of each genotype on AAO in AD patients were calculated by one-way analysis of variance and further analysis by post hoc least significant difference. All statistical analyses were performed using SPSS version 13.0 (SPSS Inc, Chicago, IL, USA). $P < 0.05$ was considered significant.

Results

General information

General information of the participants is shown in Table 1. No significant difference was found in age and sex between the two groups, while the mini-mental state examination score was significantly lower in AD. The distributions of the six common genotypes of *APOE* were under Hardy–Weinberg equilibrium in SAD patients and control subjects, respectively (Table S1).

$\epsilon 2$ allele decreased AD risk and $\epsilon 4$ allele increased AD risk

In all the subjects, the distribution of allele frequencies and genotypes of *APOE* was of significant difference between

Table 1 Characteristics of subjects in AD and control groups

	AD	Control	P
Number	875	1,195	
Age ^a (mean $\pm$ SD) (range), years	67.7 $\pm$ 9.67 (45–97)	68.5 $\pm$ 10.13 (48–94)	0.106
Male/female	397/478	493/702	0.062
MMSE (mean $\pm$ SD)	14.3 $\pm$ 6.35	28.6 $\pm$ 1.85	0.000*

Notes: * $P < 0.01$. ^aAge at onset for AD; age at entrance for control.

Abbreviations: AD, Alzheimer's disease; MMSE, mini-mental state examination; SD, standard deviation.

AD and control groups with more $\epsilon 2$, $\epsilon 3$ allele and less $\epsilon 4$ allele in controls (Table 2). There were also more $\epsilon 2$ -carrying subjects ($\epsilon 2\epsilon 2$, $\epsilon 2\epsilon 3$, and $\epsilon 2\epsilon 4$) and less $\epsilon 4$ -carrying subjects ($\epsilon 2\epsilon 4$, $\epsilon 3\epsilon 4$, $\epsilon 4\epsilon 4$) in the control group than in the AD group. When the subjects were further stratified by sex and AAO (AD with AAO ≤ 65 was defined as EOAD; AAO > 65 as late-onset AD [LOAD]), the differences remained significant (Table 2).

The impact of APOE genotype and allele frequencies on SAD risks was analyzed by binary logistic regression. As shown in Table 3, in all the subjects, $\epsilon 2\epsilon 3$ genotype decreased AD risk ($P=8\times 10^{-3}$, OR 0.64, 95% CI 0.46–0.89) while $\epsilon 2\epsilon 2$ and $\epsilon 2\epsilon 4$ genotypes did not statistically relate to AD risk. On the contrary, $\epsilon 3\epsilon 4$ and $\epsilon 4\epsilon 4$ genotype increased AD risk with dosage effect of $\epsilon 4$: the OR of $\epsilon 3\epsilon 4$ was 1.90 ($P=1.18\times 10^{-9}$, 95% CI 1.54–2.33) while the OR of $\epsilon 4\epsilon 4$ rose to 15.64 ($P=8.59\times 10^{-15}$, 95% CI 7.92–32.05). When the subjects were further divided by sex and AAO, the $\epsilon 4$ dosage effect remained constant, but the protective effect of $\epsilon 2\epsilon 3$ was significant only in the female subgroup and LOAD subgroup. As for the $\epsilon 2$ - and $\epsilon 4$ -carrying status, $\epsilon 2$ allele lowered the risk of developing AD while $\epsilon 4$ increased this risk, which existed in all subgroups after the subjects were stratified by sex and AAO, with the highest OR of 2.79 in female $\epsilon 4$ -positive subjects (Table 3).

APOE $\epsilon 4\epsilon 4$ may be associated with an earlier AAO in LOAD patients

In AD patients, only in LOAD was the AAO found to be significantly lower in $\epsilon 4$ allele-positive subjects than $\epsilon 4$ allele-negative ones (73.9 ± 5.12 vs 74.9 ± 5.18 , $P=2.2\times 10^{-2}$) (Table 4). Nevertheless, there was no difference in AAO between the $\epsilon 2$ -positive AD and $\epsilon 2$ -negative ones. We further investigated the AAO according to the dosage of $\epsilon 2$ and $\epsilon 4$. The AAO was 2 years lower in patients of $\epsilon 4\epsilon 4$ genotype than $\epsilon 4$ carriers ($\epsilon 2\epsilon 4$ and $\epsilon 3\epsilon 4$) or $\epsilon 4$ -negative ones. But no dosage effect of $\epsilon 4$ on AAO was found.

We further investigated the effect of APOE $\epsilon 2$ and $\epsilon 4$ haplotype on AD risk in different AAO and found $\epsilon 2$'s protective role against AD in the patients with AAO of 61–65 and above 76 (Table 5). The $\epsilon 4$ haplotype increased AD risk in patients with AAO below 55 and 61–75 with the highest OR of 3.842 in 66–70 groups. The risk decreased when AAO was above 76, though there was no significant difference.

Discussion

In 1993, APOE $\epsilon 4$ was first reported to increase SAD risks and advance AAO of AD in a gene dosage way.^{1,2,16} Except

Table 2 Distribution of allele frequencies and genotypes of APOE in AD and controls

	APOE	Numbers	Genotypes (%)			Allele frequencies (%)							
			Genotype	$\epsilon 2\epsilon 2$	$\epsilon 2\epsilon 3$	$\epsilon 2\epsilon 4$	$\epsilon 3\epsilon 3$	$\epsilon 3\epsilon 4$	$\epsilon 4\epsilon 4$	$\epsilon 2$	$\epsilon 3$	$\epsilon 4$	$\epsilon 2$ (+)
Total	AD (%)	875	0.5	6.2	2.5	48.5	33.0	9.4	4.8	68.1	27.1	9.1	44.9
	Control (%)	1,195	0.6	12.3	3.4	60.9	22.0	0.8	8.5	78.1	13.5	16.3	26.2
	χ^2 (P)		$143.5\ (3.15\times 10^{-29})^*$						$131.3\ (3.08\times 10^{-29})^*$			$22.6\ (2.02\times 10^{-6})^*$	$78.8\ (6.94\times 10^{-19})^*$
Male	AD	397	0.3	7.1	2.3	51.6	31.2	7.6	4.9	70.8	24.3	9.6	41.1
	Control	493	0.6	11.6	4.5	59.8	22.7	0.8	8.6	77.0	14.4	16.6	28.0
	χ^2 (P)		$43.2\ (3.39\times 10^{-9})^*$						$33.9\ (4.37\times 10^{-8})^*$			$9.4\ (0.002)^*$	$16.8\ (4.21\times 10^{-5})^*$
Female	AD	478	0.6	5.4	2.7	45.8	34.5	10.7	4.7	65.8	29.5	8.8	48.1
	Control	702	0.6	12.8	2.7	61.7	21.5	0.7	8.3	78.8	12.8	16.1	24.9
	χ^2 (P)		$107.5\ (1.35\times 10^{-21})^*$						$104.9\ (1.82\times 10^{-23})^*$			$13.3\ (2.63\times 10^{-4})^*$	$67.8\ (1.78\times 10^{-16})^*$
EOAD	AD	354	0	6.5	2.8	49.7	32.5	8.5	4.7	69.2	26.1	9.3	43.8
	Control	483	0.8	11.4	4.6	60.9	21.7	0.6	8.8	77.4	13.8	16.8	26.9
	χ^2 (P)		$55.2\ (1.17\times 10^{-10})^*$						$46.5\ (7.88\times 10^{-11})^*$			$9.6\ (0.002)^*$	$25.9\ (3.61\times 10^{-7})^*$
LOAD	AD	521	0.8	6.0	2.3	47.6	33.4	10.0	4.9	67.3	27.8	9.0	45.7
	Control	712	0.4	12.9	2.7	61.0	22.2	0.8	8.2	78.5	13.3	16.0	25.7
	χ^2 (P)		$92.6\ (1.92\times 10^{-18})^*$						$85.7\ (2.45\times 10^{-19})^*$			$13.0\ (3.20\times 10^{-4})^*$	$53.4\ (2.71\times 10^{-13})^*$

Note: * $P<0.01$.

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E gene; EOAD, early-onset AD; LOAD, late-onset AD.

Table 3 Logistic regression of *APOE* genotypes and allele frequencies in Alzheimer's disease patients and controls

Total	$\epsilon 2\epsilon 2$	$\epsilon 2\epsilon 3$	$\epsilon 2\epsilon 4$	$\epsilon 3\epsilon 3$	$\epsilon 3\epsilon 4$	$\epsilon 4\epsilon 4$	$\epsilon 2 (+)$	$\epsilon 4 (+)$
P	0.994	$8 \times 10^{-3*}$	0.682	1.ref	$1.18 \times 10^{-9*}$	$8.59 \times 10^{-15*}$	$2.88 \times 10^{-6*}$	$1.57 \times 10^{-18*}$
OR	1.01	0.64	0.90		1.90	15.93	0.52	2.30
95% CI	0.29–3.46	0.46–0.89	0.53–1.53		1.54–2.33	7.92–32.05	0.39–0.68	1.91–2.77
Male								
P	0.50	0.22	0.16	1.ref	$4 \times 10^{-3*}$	$8.87 \times 10^{-6*}$	$6 \times 10^{-3*}$	$7.22 \times 10^{-5*}$
OR	0.46	0.735	0.561		1.58	11.06	0.54	1.77
95% CI	0.05–4.42	0.45–1.20	0.25–1.25		1.16–2.16	3.83–31.94	0.36–0.81	1.34–2.34
Female								
P	0.61	$1.8 \times 10^{-2*}$	0.41	1.ref	$3.79 \times 10^{-8*}$	$2.02 \times 10^{-10*}$	$3.19 \times 10^{-4*}$	$4.61 \times 10^{-16*}$
OR	1.48	0.57	1.36		2.16	20.58	0.50	2.79
95% CI	0.33–6.66	0.36–0.91	0.66–2.80		1.64–2.84	8.10–52.27	0.35–0.73	2.18–3.58
EOAD								
P	1.00	0.25	0.45	1.ref	$4.17 \times 10^{-4*}$	$1.91 \times 10^{-6*}$	$3 \times 10^{-3*}$	$6.73 \times 10^{-7*}$
OR	0.00	0.74	0.74		1.80	18.83	0.52	2.10
95% CI	0	0.44–1.24	0.34–1.61		1.30–2.50	5.63–63.03	0.34–0.80	1.57–2.81
LOAD								
P	0.28	$2.3 \times 10^{-2*}$	0.82	1.ref	$1.18 \times 10^{-6*}$	$1.25 \times 10^{-9*}$	$1.0 \times 10^{-3*}$	$9.73 \times 10^{-13*}$
OR	2.30	0.60	1.09		1.94	14.41	0.53	2.41
95% CI	0.51–10.41	0.39–0.93	0.52–2.28		1.48–2.53	6.09–34.08	0.37–0.76	1.89–3.07

Note: * $P < 0.01$.

Abbreviations: *APOE*, apolipoprotein E gene; CI, confidence interval; EOAD, early-onset Alzheimer's disease; LOAD, late-onset Alzheimer's disease; OR, odds ratio.

for several investigations,¹⁷ most studies confirmed the results.^{18–23} The dose effect of *APOE* $\epsilon 4$ on AD risk was reported to be ascribed to increased A β , A β oligomers, and plaque deposition and reduced metabolism in certain parts of the brain.²⁴

The allele frequencies and genotypes vary among different ethnic groups.²⁵ In our study, the *APOE* allele frequencies (AD: $\epsilon 2$ 4.8%, $\epsilon 3$ 68.1%, and $\epsilon 4$ 27.1%; control $\epsilon 2$ 8.5%, $\epsilon 3$ 78.1%, and $\epsilon 4$ 13.5%) were similar to the *APOE* survey in Shanghai consisting of 65 AD patients and 363 cognitively normal controls (AD: $\epsilon 2$ 4.6%, $\epsilon 3$ 70%, and $\epsilon 4$ 25.4%; control: $\epsilon 2$ 8.6%, $\epsilon 3$ 80.4%, and $\epsilon 4$ 11%).⁶ We also found that

the *APOE* $\epsilon 4$ was the independent risk factor of AD and increased the AD risk in a gene dosage way. This is quite consistent with the investigations in other populations.^{7,26,27} However, the ORs in $\epsilon 3\epsilon 4$ genotype toward AD risks were relatively smaller in all the subjects and subgroups of LOAD, EOAD, male, and female (1.579–2.159) groups, compared with those reported in other studies^{7,21,25,28} (usually > 2). The ORs in $\epsilon 4\epsilon 4$ genotype ranged from 11.061 to 20.581, much greater than those of $\epsilon 3\epsilon 4$ and in accordance with the results in other studies. The genotype of $\epsilon 2\epsilon 4$ showed a risk factor of AD in some studies,⁷ but did not show any protective or risk effect on AD pathogenesis in our study, which might

Table 4 The *APOE* $\epsilon 2$ and $\epsilon 4$ allele dosage effect on age at onset in AD patients

	$\epsilon 2 (+)$		$\epsilon 2 (-)$		No $\epsilon 2$		One $\epsilon 2$		Two $\epsilon 2$	
Total AAO, n	67.7 $\pm$ 9.60	80	67.7 $\pm$ 9.69	795	67.7 $\pm$ 9.69	795	67.5 $\pm$ 9.75	76	72.0 $\pm$ 4.97	4
EOAD AAO, n	57.9 $\pm$ 4.30	33	57.7 $\pm$ 5.09	321	57.7 $\pm$ 5.01	321	57.9 $\pm$ 4.30	33	0	0
LOAD AAO, n	74.6 $\pm$ 5.30	47	74.4 $\pm$ 5.17	474	74.4 $\pm$ 5.17	474	74.9 $\pm$ 5.32	43	72.0 $\pm$ 4.97	4
Male AAO, n	67.0 $\pm$ 9.24	38	67.6 $\pm$ 9.77	359	67.6 $\pm$ 9.77	359	66.7 $\pm$ 9.18	37	78.0	1
Female AAO, n	68.4 $\pm$ 9.98	42	67.8 $\pm$ 9.63	436	67.8 $\pm$ 9.62	436	68.3 $\pm$ 10.32	39	70.0 $\pm$ 9.64	3
	$\epsilon 4 (+)$		$\epsilon 4 (-)$		No $\epsilon 4$		One $\epsilon 4$		Two $\epsilon 4$	
Total AAO, n	67.9 $\pm$ 9.80	706	68.2 $\pm$ 10.01	1,364	67.8 $\pm$ 9.95	482	67.6 $\pm$ 9.69	311	67.4 $\pm$ 7.88	82
EOAD AAO, n	58.0 $\pm$ 5.13	155	57.6 $\pm$ 4.93	199	57.6 $\pm$ 4.93	199	57.6 $\pm$ 5.20	125	59.2 $\pm$ 4.72	30
LOAD AAO, n	73.9 $\pm$ 5.12	238	74.9 $\pm$ 5.18 ^a	283	74.9 $\pm$ 5.18	283	74.4 $\pm$ 5.10	186	72.2 $\pm$ 4.86 ^b	52
Male AAO, n	67.8 $\pm$ 9.58	163	67.3 $\pm$ 9.82	234	67.3 $\pm$ 9.81	234	67.6 $\pm$ 9.85	133	68.6 $\pm$ 8.38	30
Female AAO, n	67.5 $\pm$ 9.17	230	68.2 $\pm$ 10.08	248	68.2 $\pm$ 10.08	248	67.7 $\pm$ 9.59	178	66.7 $\pm$ 7.57	52

Notes: ^aDifference between AAO in LOAD in $\epsilon 4 (+)$ and $\epsilon 4 (-)$ status was significant ($P=0.022$). ^bDifference between AAO in LOAD in carriers of one and two *APOE* $\epsilon 4$ alleles was significant ($P=0.006$).

Abbreviations: AD, Alzheimer's disease; AAO, age at onset; *APOE*, apolipoprotein E gene; EOAD, early-onset AD; LOAD, late-onset AD.

Table 5 Effect of APOE $\epsilon 2$ and $\epsilon 4$ haplotype on AD risk stratified by age

	AAO	AD (%)	Control (%)	P-value	OR (95% CI)
$\epsilon 2$ (+)	≤ 55	9 (8.0)	26 (15.8)	0.088	0.492 (0.218–1.110)
	56–60	15 (11.7)	20 (15.2)	0.572	0.810 (0.391–1.680)
	61–65	9 (7.9)	35 (18.8)	0.016*	0.382 (0.175–0.833)
	66–70	13 (10.0)	20 (12.9)	0.422	0.737 (0.349–1.554)
	71–75	16 (8.7)	6 (14.6)	0.062	0.552 (0.296–1.030)
	76–80	11 (7.9)	31 (18.6)	0.009**	0.376 (0.181–0.781)
	> 80	7 (10.3)	27 (18.9)	0.027*	0.888 (0.800–0.986)
$\epsilon 4$ (+)	≤ 55	51 (45.5)	49 (29.7)	0.010*	1.954 (1.173–3.256)
	56–60	49 (38.3)	35 (26.5)	0.051	1.700 (0.998–2.896)
	61–65	55 (48.2)	46 (24.7)	5.13×10^{-5} **	2.817 (1.706–4.650)
	66–70	73 (56.2)	39 (25.2)	1.59×10^{-7} **	3.842 (2.323–6.355)
	71–75	84 (45.7)	54 (21.9)	2.99×10^{-7} **	3.011 (1.975–4.590)
	76–80	55 (39.6)	53 (31.7)	0.149	1.417 (0.883–2.276)
	> 80	26 (38.2)	37 (25.9)	0.073	1.772 (0.948–3.314)

Notes: * $P < 0.05$; ** $P < 0.01$.

Abbreviations: AAO, age at onset; AD, Alzheimer's disease; APOE, apolipoprotein E gene; CI, confidence interval; OR, odds ratio.

be due to the small frequency of $\epsilon 2\epsilon 4$ genotypes or the co-existence of a “protective” and harmful effect in $\epsilon 2$ allele and $\epsilon 4$ allele, respectively.

In the present investigation, the APOE $\epsilon 2$ decreased the AD risks, and $\epsilon 2\epsilon 3$ lowered the AD risk in total population, female, and LOAD subgroups. This effect is similar to those observed in other populations.^{27,29} But the “protective” effect did not always show due to the lower frequency of $\epsilon 2$ allele.^{7,30} In one study of north Chinese population, the $\epsilon 2$ was indicated as a protective factor in male population, which was contrary to our result. This might be contributed to their small subject number and imbalanced sex distribution between AD and control groups.¹²

Researches indicated that the $\epsilon 4$ allele took part in the pathogenesis of EOAD as well,^{29,31} and it was well replicated in our study. But a previous report in Chinese population did not find any association, which could be due to the small number of subjects.¹² Some investigations indicated that $\epsilon 4$ increased AD risk in women more than men,^{32,33} but others did not find the pattern.^{21,29} In a prospective study in Latin Americans, APOE $\epsilon 4$ allele risk was significant only in women,³² but had a stronger effect in men from Sweden and Finland.^{27,34} In our population, APOE $\epsilon 4$ increased AD risk in both sexes with a higher OR in the female group. The different results might be caused by ethnic origins.

APOE $\epsilon 2$ allele was reported not to affect AAO in AD patients in most studies.³¹ The differences of AAO between $\epsilon 2\epsilon 2$ group and one or no $\epsilon 2$ group did not reach significant, though the AAO of $\epsilon 2\epsilon 2$ was 4 years later in number than that of one $\epsilon 2$ group. This might be attributed to the very low frequencies of $\epsilon 2\epsilon 2$ genotypes in AD. APOE $\epsilon 4$ allele was reported to lower the AAO of LOAD in a gene dosage

way. The AAO of AD patients with APOE $\epsilon 4\epsilon 4$ genotype was 5–16 years lower than those with $\epsilon 4$ -negative ones.¹⁶ In our subjects, the AAO had a decrease of 2 years in patients of $\epsilon 4\epsilon 4$ genotype in contrast to $\epsilon 4$ carriers ($\epsilon 2\epsilon 4$ and $\epsilon 3\epsilon 4$) or $\epsilon 4$ -negative ones, but not in a gene dosage way. Though we found some significant differences in AAO in the current study, we still could not deduce the effect of APOE genotype on the AAO with respect to the cross-sectional study. So further prospective studies should be implemented in Chinese Han population.

Whether APOE $\epsilon 2$ would reduce AD risk in a certain AAO range was controversial.^{27,35} We found $\epsilon 2$'s protective role in the AAO of 61–65 and above 76. As for $\epsilon 4$ haplotype, its AD risk decreased in people aged 70 years and above,³⁶ which was quite similar to our results. There might be other genetic risk factors or environmental effects contributing to the AD onset in very old people.

This study had a few limitations. First, factors like diabetes, history of depression, stroke, and heart attack may also contribute to AD pathogenesis, but due to the incomplete information, we did not put those covariants into the binary logistic regression. Second, the subjects were recruited from memory disorders clinics but not the general population, which might exaggerate the impact of APOE $\epsilon 4$ on AD risks. In the population-based studies, the positive predictive value was lower, and hence APOE $\epsilon 4$ is not recommended for a screen test for AD.^{22,34}

In conclusion, the APOE $\epsilon 4$ allele is a strong risk factor in AD in the Chinese Han population and it affects AD risk in a gene dosage effect, similar to those in other populations. The risk of APOE $\epsilon 3\epsilon 4$ toward AD was relatively smaller and the APOE $\epsilon 4\epsilon 4$ genotype lowered the AAO of AD in the

LOAD group, which might be the specific characteristics of *APOE* polymorphisms in the Chinese Han population. The role of *APOE* protein in AD pathogenesis and other loci that will help to increase the AD-predictive value combined with *APOE* $\epsilon 4$ should be studied in the future.

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Author contributions

Ping Wu was responsible for data collection, data analysis, and manuscript drafting. Yi-Min Sun was responsible for study design, data analysis, data interpretation, and editing the manuscript. Hong-Lei Li, Zhi-Jun Liu, Qing-Qing Tao, Miao Xu, Qi-Hao Guo, and Zhen Hong were responsible for study implementation and data collection. All authors contributed equally to revising the manuscript and have approved the final version.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Hardy–Weinberg equilibrium of APOE in AD patients and controls

APOE	AD (actual)	AD (expected)	Controls (actual)	Controls (expected)
Number	875 (%)		1,195 (%)	
$\epsilon 2\epsilon 2$	4 (0.5)	2 (0.2)	7 (0.6) 1.0	9 (0.8)
$\epsilon 2\epsilon 3$	54 (6.2)	57 (6.5)	147 (12.3)	158 (13.2)
$\epsilon 2\epsilon 4$	22 (2.5)	22 (2.5)	41 (3.4)	27 (2.3)
$\epsilon 3\epsilon 3$	424 (48.5)	406 (46.5)	728 (60.9)	728 (60.9)
$\epsilon 3\epsilon 4$	289 (33.0)	322 (36.9)	263 (22.0)	251 (21.0)
$\epsilon 4\epsilon 4$	82 (9.4)	64 (7.3)	9 (0.8)	22 (1.8)
$\epsilon 2$	84 (4.8)		202 (8.5)	
$\epsilon 3$	1,191 (68.1)		1,866 (78.1)	
$\epsilon 4$	475 (27.1)		322 (13.5)	
$\epsilon 2 (P)$	5.137 (0.399)		9.261 (0.099)	

Abbreviations: AD, Alzheimer's disease; APOE, apolipoprotein E gene.

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