

## RESEARCH ARTICLE

# The association between the ApoE polymorphisms and the MRI-defined intracranial lesions in a cohort of southern China population

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## Abstract

**Background:** The apolipoprotein E (APOE)  $\epsilon 4$  allele is considered as a risk factor for Alzheimer's disease (AD). However, the association of APOE allele with MRI evidence of intracranial lesions has not been well understood.

**Methods:** Quantitative real-time PCR was performed to detect the APOE genotype; MRI was examined for intracranial lesions. Their association was evaluated in a cohort of 226 AD patients and 2607 healthy individuals in southern China.

**Results:** The frequencies of  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  alleles were 8.0%, 82.9%, and 9.1% in the whole study population. The frequency of APOE- $\epsilon 4$  allele was significantly higher in the AD subjects than that in the control group (14.4% vs 8.6%,  $P < 0.001$ ). We found that brain atrophy occurred at a rate of 12.3% in  $\epsilon 4$  allele group vs 8.5% in non- $\epsilon 4$  genotype group, with a significance of  $P = 0.008$ . Severe brain atrophy occurred at a rate of 1.0% in  $\epsilon 4$  allele group vs 0.2% in non- $\epsilon 4$  genotype group ( $P = 0.011$ ). The individuals carrying APOE  $\epsilon 4/\epsilon 4$  had an odds ratio (OR) of 7.64 ( $P < 0.01$ ) for developing AD, while the APOE  $\epsilon 3/\epsilon 4$  gene carriers had an OR of 1.47 ( $P = 0.031$ ) and the OR in APOE  $\epsilon 2/\epsilon 3$  carriers is 0.81 ( $P = 0.372$ ). Interestingly, we found that the risk of  $\epsilon 4/\epsilon 4$  allele carrier developing AD was significantly higher in male ( $P < 0.001$ ) than female ( $P = 0.478$ ).

**Conclusion:** Compared to  $\epsilon 2$  and  $\epsilon 3$  alleles, the presence of APOE- $\epsilon 4$  allele might increase the risk for AD in a dose-dependent manner in southern China. Moreover, the presence of APOE- $\epsilon 4$  allele results in a higher incidence of brain atrophy.

## KEYWORDS

apolipoprotein E gene, brain atrophy, gene polymorphisms, magnetic resonance imaging

Zhuoran Li, Na Yang, and Xiuxia Lei contributed equally and share the first authorship.

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## 1 | INTRODUCTION

Alzheimer's disease (AD) is a progressive brain disorder that results in loss of memory, associated with unusual behavior, personality changes, and an irreversible decline in cognitive skills.<sup>1-3</sup> As a matter of fact, AD is the most common form of dementia in elderly people worldwide. Approximately 13% of people over the age of 65 years and 45% over the age of 85 years are subjected to AD.<sup>4</sup> More worse, the number of affected people is estimated to be doubled in the next 20 years.<sup>5,6</sup> Thus, accurate prediction and early prevention of AD are of great importance for reducing and postponing the occurrence of the disease.

Evidences from different populations show that the apolipoprotein E (APOE) genetic polymorphisms were associated with the risk of AD occurrence.<sup>4,7-9</sup> It is well known that APOE is a 299-amino acid protein that acts as a major lipid transporter abundantly found in the brain.<sup>10</sup> There are three major APOE polymorphic alleles in humans, including APO- $\epsilon$ 2, APO- $\epsilon$ 3, and APO- $\epsilon$ 4, which encode three protein isoforms of APOE2, APOE3, and APOE4. There are only two amino acid cysteine/arginine polymorphisms at positions 112 or 158 in the N-terminal domain among the three isoforms. Although the  $\epsilon$ 4 allele of the APOE4 is considered as a genetic susceptibility for AD in diverse ethnic groups,<sup>11-17</sup> the frequencies of the three APOE alleles were found not to be the same for all the ethnic groups in the world.

Nowadays, magnetic resonance imaging (MRI) has been widely used for imaging modality in AD diagnosis.<sup>18</sup> It can be used to characterize the structural changes in the brain and classify the different imageological changes of AD such as cerebral infarction and encephalatrophy.<sup>19,20</sup> More important, MRI atrophy measures are regarded as predictive biomarkers for AD, indicating the disease's state and its progression.<sup>21</sup> Herein, we examined the relationship between APOE genetic polymorphisms and MRI imageological changes in a cohort of AD patients and cognitively normal subjects every year. We next evaluated the APOE genotypes and allele frequencies and their contribution to the development of AD in a large population in southern China. We also provided the clinical evidences of using ApoE- $\epsilon$ 4-targeted therapeutic strategy to abate the neurodegeneration of Alzheimer's pathogenesis.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

The study consisted of 226 AD patients and 2607 healthy individuals—as a control group, all ethnic Chinese. Here, 274 healthy individuals from control group were selected with no age difference against the AD group. These subjects were recruited from the geriatric department of Guangzhou First People's Hospital during the period ranging from October 2016 to March 2018, approved by the Ethics Committee of Guangzhou First People's Hospital in accordance with the Helsinki Declaration. Blood samples were drawn from each subject after an informed consent from the subject or from his/her legal

guardian. The whole study population included 1663 males and 1170 females, aged  $70.8 \pm 14.9$  years old. Neurological examination was conducted by one neurologist in all patients and included the Mini-Mental State Examination (MMSE) and clock-drawing test. The diagnoses of AD were based upon the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) clinical diagnostic criteria (Mckhann et al and DSM IV 1994), MRI.

### 2.2 | APOE genotyping

For each subject, 2 mL peripheral blood exposed to EDTA anticoagulant was collected. Genomic DNA was extracted from peripheral blood leukocytes using modified phenol-chloroform extraction method. The ApoE gene was detected by using a fluorescent probe-based PCR kit (YZYMT-011, Wuhan YZY Medical Science and Technology Co., Ltd.). The Real-time PCR Protocol was carried out on a Real-Time PCR Instrument (ABI7500, Life Technologies Holdings Pte Ltd.) as follows: 37°C for 10 minutes, 94°C for 5 minutes for denaturation, 40 cycles of denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 60 seconds. There are two main types of ApoE gene: 526 C > T and 388 T > C single nucleotide polymorphism. The APOE gene was divided into six genotypes:  $\epsilon$ 2/ $\epsilon$ 2,  $\epsilon$ 2/ $\epsilon$ 3,  $\epsilon$ 2/ $\epsilon$ 4,  $\epsilon$ 3/ $\epsilon$ 3,  $\epsilon$ 3/ $\epsilon$ 4, and  $\epsilon$ 4/ $\epsilon$ 4.

### 2.3 | Brain MRI examination

MRI scanning was performed on a 3-Tesla MRI scanner (Siemens) and an 8-channel brain array coil. Participant head movement was minimized using foam padding, and headphones were used to reduce scanner noise. MRI scans were obtained with T1WI, T2WI, and T2 FLAIR imaging sequence (FOV = 253 mm  $\times$  230 mm, SW = 6 mm, matrix = 64  $\times$  58). All subjects underwent a MRI scan after being instructed to relax with their eyes closed but avoid falling asleep.

### 2.4 | Image interpretation

MRI data were analyzed by blind method by two senior MR diagnostic physicians. The visual evaluation method was used to observe the structural abnormality in all brain regions, and the lateral ventricle enlargement and the widening of cerebral sulcus were used as the visual evaluation criteria for brain atrophy.

### 2.5 | Statistical analysis

Genotype and allele frequencies of APOE were compared between the AD and the control cases using the chi-square test and Fisher's exact test when appropriate. All odds ratios (ORs) involving genotypes were calculated by logistic regression adjusted for gender. Two-tailed Student's *t* test and ANOVA were used to compare quantitative data. The SPSS statistical software package version 11.5 was used for all the statistical analysis.  $P < 0.05$  was considered as statistical significance.

### 3 | RESULT

#### 3.1 | The frequency of APOE- $\epsilon$ 4 allele was higher in the AD patients than that in the healthy

The frequencies of APOE gene are presented in Table 1. Gender, age, genotype, and allele frequency of APOE were compared between the AD and the control groups. Here, 226 AD patients and 2607 control individuals were included. Of all the population, 1170 were female (41.3%) and 1663 were male (58.7%). The frequency of APOE- $\epsilon$ 4 allele in the AD group was significantly higher than that of the control group (14.4% vs 8.6%;  $P < 0.001$ ). The allele frequencies of  $\epsilon$ 2,  $\epsilon$ 3, and  $\epsilon$ 4 alleles were 6.2%, 79.4%, and 14.4% in AD patients, and the cases in control group were 8.2%, 83.2%, and 8.6%, respectively. No significant difference was found in the APOE- $\epsilon$ 2 allele frequencies between the two groups ( $P = 0.141$ ), whereas there was a significant difference between the two groups in the APOE- $\epsilon$ 3 allele frequencies ( $P = 0.041$ ). Notably, there was a significant difference between the AD group and the control group in the genotype of  $\epsilon$ 3/ $\epsilon$ 4 ( $P = 0.03$ ) and  $\epsilon$ 4/ $\epsilon$ 4 ( $P < 0.001$ ). Our results showed that 25.2% of the AD patients had one or two APOE  $\epsilon$ 4 alleles, but only 16.8% of the control individuals carried an APOE  $\epsilon$ 4 allele. To exclude the influence of age, we further compared a cohort of 226 AD patients and 274 control individuals which had no significant difference in age. As shown in Table 2, a significant difference in the APOE- $\epsilon$ 4 allele frequencies (14.4% vs 8.8%,  $P = 0.005$ ) remains between the two groups.

#### 3.2 | The association between APOE- $\epsilon$ 4 allele and brain imaging

As shown in Table 3, Fisher's exact test was used to compare the MR imaging change between APOE- $\epsilon$ 4 allele group and non- $\epsilon$ 4 allele group. Brain atrophy occurred at a rate of 12.3% in  $\epsilon$ 4 allele group

**TABLE 1** The distribution of ApoE genotypes and alleles in AD patients and control subjects

	AD patients (n = 226)	Control subjects (n = 2607)	P-value
Age (y)	83.3 ± 8.2	69.7 ± 14.8	<0.001
Sex (M/F)	133/93	1530/1077	0.962
$\epsilon$ 2/ $\epsilon$ 2	1 (0.4%)	19 (0.7%)	0.518
$\epsilon$ 2/ $\epsilon$ 3	23 (10.2%)	351 (13.5%)	0.161
$\epsilon$ 2/ $\epsilon$ 4	3 (1.3%)	36 (1.4%)	0.621
$\epsilon$ 3/ $\epsilon$ 3	145 (64.2%)	1799 (69.0%)	0.132
$\epsilon$ 3/ $\epsilon$ 4	46 (20.4%)	389 (14.9%)	0.03
$\epsilon$ 4/ $\epsilon$ 4	8 (3.5%)	13 (0.5%)	<0.001
$\epsilon$ 2	28 (6.2%)	425 (8.2%)	0.141
$\epsilon$ 3	359 (79.4%)	4338 (83.2%)	0.041
$\epsilon$ 4	65 (14.4%)	451 (8.6%)	<0.001

Note: Mean ± SD.

Abbreviation: AD: Alzheimer's disease.

vs 8.5% in non- $\epsilon$ 4 genotype group ( $P = 0.008$ ), indicating a significant difference. Severe brain atrophy occurred at a rate of 1.0% in  $\epsilon$ 4 allele group vs 0.2% in non- $\epsilon$ 4 genotype group ( $P = 0.011$ ). The results suggest that the population carrying APOE- $\epsilon$ 4 gene had a higher incidence of brain atrophy and severe brain atrophy in the entire study population. However, there was no significant difference in the incidence of cerebral infarction and cerebral infarction with brain atrophy between the two groups. There existed a

**TABLE 2** The distribution of ApoE genotypes and alleles in AD patients and 274 control subjects

	AD patients (n = 226)	Control subjects (n = 274)	P-value
Age (y)	83.3 ± 8.2	82.4 ± 8.7	0.064
Sex (M/F)	133/93	161/113	0.984
$\epsilon$ 2/ $\epsilon$ 2	1 (0.4%)	2 (0.7%)	0.572
$\epsilon$ 2/ $\epsilon$ 3	23 (10.2%)	38 (13.9%)	0.209
$\epsilon$ 2/ $\epsilon$ 4	3 (1.3%)	4 (1.5%)	0.605
$\epsilon$ 3/ $\epsilon$ 3	145 (64.2%)	187 (68.2%)	0.335
$\epsilon$ 3/ $\epsilon$ 4	46 (20.4%)	42 (15.3%)	0.142
$\epsilon$ 4/ $\epsilon$ 4	8 (3.5%)	1 (0.4%)	0.009
$\epsilon$ 2	28 (6.2%)	46 (8.4%)	0.186
$\epsilon$ 3	359 (79.4%)	454 (82.8%)	0.167
$\epsilon$ 4	65 (14.4%)	48 (8.8%)	0.005

Note: Mean ± SD.

Abbreviation: AD: Alzheimer's disease.

**TABLE 3** Comparison of MRI imaging change and the distribution of age and gender between non- $\epsilon$ 4 and  $\epsilon$ 4 allele groups in whole population

	$\epsilon$ 4 (n = 495)	Non- $\epsilon$ 4 (n = 2338)	P value
Age (y)	70.14 ± 15.0	70.9 ± 14.8	0.227
Sex (M/F)	300/195	1363/975	0.343
Cerebral infarction	212 (42.8%)	949 (40.6%)	0.358
Age (y)	76.1 ± 13.0	77.0 ± 12.4	0.298
Sex (M/F)	140/72	636/313	0.784
Brain atrophy	61 (12.3%)	199 (8.5%)	0.008*
Age (y)	80.2 ± 8.9	78.1 ± 9.8	0.134
Sex (M/F)	42/19	107/92	0.037*
Cerebral infarction with brain atrophy	44 (8.9%)	129 (5.5%)	0.044*
Age (y)	81.3 ± 9.2	79.9 ± 10.1	0.440
Sex (M/F)	32/12	80/49	0.199
Severe brain atrophy	5 (1.0%)	4 (0.2%)	0.011*
Age (y)	81.6 ± 11.1	87.0 ± 1.0	0.447
Sex (M/F)	3/2	3/1	0.714

\*Significant difference.

significant difference in the incidence of brain atrophy (12.3% vs 8.5%,  $P = 0.008$ ), severe brain atrophy (1.0% vs 0.2%,  $P = 0.011$ ), and cerebral infarction with brain atrophy (8.9% vs 5.5%,  $P = 0.044$ ) between  $\epsilon 4$  allele group and non- $\epsilon 4$  allele group, while no significant difference was found in cerebral infarction. In respect to the distribution of age, there was no significant difference between the  $\epsilon 4$  allele group and non- $\epsilon 4$  allele group. In the case of patients with brain atrophy, there was a significant difference in the distribution of gender between the non- $\epsilon 4$  and  $\epsilon 4$  allele group ( $P = 0.037$ ), while no significant difference was found in cerebral infarction and cerebral infarction with brain atrophy. Table 4 shows a comparison of MRI imageological change between non- $\epsilon 4$  and  $\epsilon 4$  allele group in AD patients. There was a significant difference in the incidence of severe brain atrophy (8.8% vs 1.8%,  $P = 0.026$ ) between  $\epsilon 4$  allele group and non- $\epsilon 4$  allele group, while no significant difference was found in brain atrophy, cerebral infarction, and cerebral infarction with brain atrophy. As shown in Table 5, no difference was found between  $\epsilon 4$  allele and non- $\epsilon 4$  allele group in all brain image changes among non-AD population.

Figure 1 shows the representative MR imaging results of the AD patients with (Figure 1A,B) and without (Figure 1C) APOE $\epsilon 4$  allele. The AD patients carrying  $\epsilon 4$  allele generally showed brain atrophy and severe brain atrophy which are accompanied by lacunar infarction, bilateral radiant crown, and bilateral basal ganglia. The hippocampal can be seen with different degrees of atrophy, which is characterized by a flattened hippocampal formation, a widening of the lateral ventricle, and an enlarged space of the cerebral effusion. The brain atrophy and hippocampus atrophy are also seen in AD patients without carrying the  $\epsilon 4$  gene, but this case is not so obvious

**TABLE 4** Comparison of MRI imaging change between non- $\epsilon 4$  and  $\epsilon 4$  allele groups in AD patients

	$\epsilon 4$ (n = 57)	Non- $\epsilon 4$ (n = 169)	P value
Age (y)	82.8 ± 7.3	83.5 ± 8.5	0.561
Sex (M/F)	33/24	100/69	0.865
Cerebral infarction	37 (64.9%)	117 (69.2%)	0.545
Age (y)	84.8 ± 6.4	85.2 ± 7.1	0.779
Sex (M/F)	23/14	75/42	0.831
Brain atrophy	37 (64.9%)	94 (55.6%)	0.219
Age (y)	81.8 ± 6.3	82.8 ± 7.5	0.471
Sex (M/F)	24/13	49/45	0.186
Cerebral infarction with brain atrophy	24 (42.1%)	65 (38.5%)	0.626
Age (y)	83.5 ± 5.8	85.0 ± 6.4	0.343
Sex (M/F)	17/7	40/25	0.417
Severe brain atrophy	5 (8.8%)	3 (1.8%)	0.026*
Age (y)	81.6 ± 11.1	87.0 ± 1.0	0.447
Sex (M/F)	3/2	2/1	0.714

\*Significant difference.

as compared to the patients carrying the  $\epsilon 4$  gene (Figure 1C). No abnormalities in hippocampal morphology or volume were observed in patients with non-AD (Figure 1D).

As shown in Table 6, 154 out of 226 cases (68.1%) of AD patients developed cerebral infarction; 1007 out of 2607 cases (38.6%) of non-AD patients developed cerebral infarction. There was a significant difference in the incidence between the two groups ( $\chi^2 = 52.39$ ,  $P < 0.001$ ). And the incidence of cerebral infarction in AD group was also higher than the control group with 274 non-AD patients ( $\chi^2 = 47.38$ ,  $P < 0.001$ ) (Table 7). These results suggested that AD patients had a higher risk to develop cerebral infarction than control group.

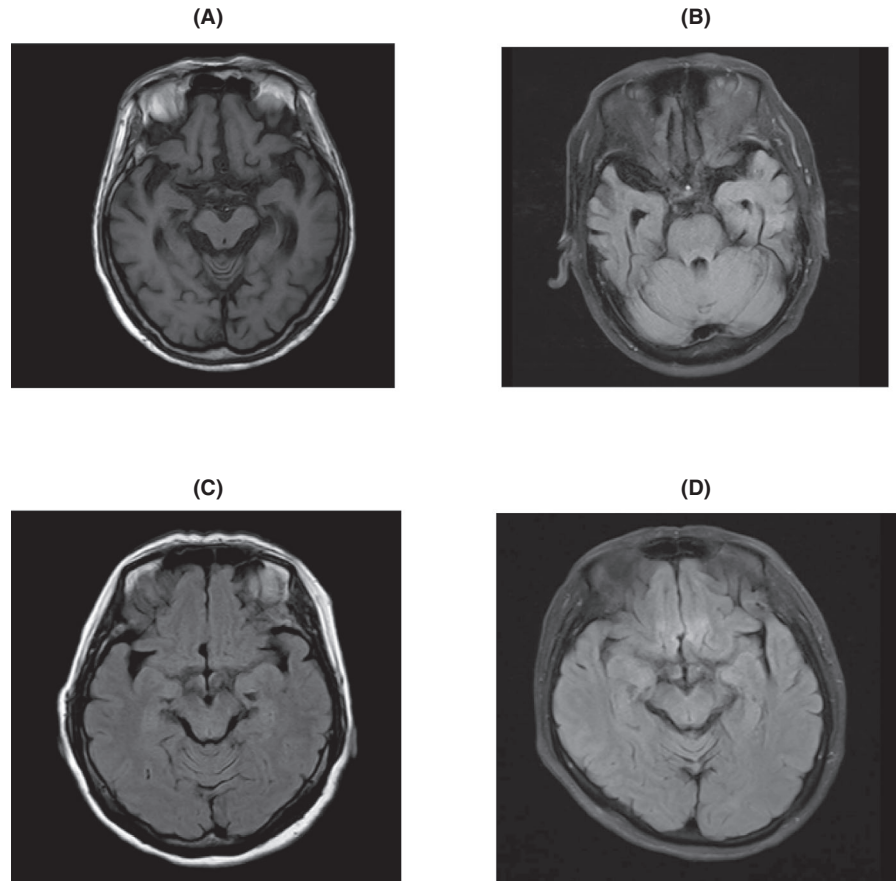
### 3.3 | APOE- $\epsilon 4$ allele conferred higher risk for AD in male than female

Table 8 shows the odds ratio of ApoE genotypes and alleles with reference to  $\epsilon 3/\epsilon 3$  in the female and male of AD, control groups, and entire population. An increased risk for AD was found both in homozygous and heterozygous carriers of the APOE- $\epsilon 4$  allele. In entire population, APOE  $\epsilon 4/\epsilon 4$  carriers had an OR of 7.64 ( $P < 0.01$ ) for developing AD, while APOE  $\epsilon 3/\epsilon 4$  carriers had an OR of 1.47 ( $P = 0.031$ ) and APOE  $\epsilon 2/\epsilon 3$  carriers had an OR of 0.81 ( $P = 0.372$ ). Notably, the ORs of the female and male with two copies of the APOE- $\epsilon 4$  alleles were 2.16 (95% CI = 0.26-18.27,  $P = 0.478$ ) and 12.0 (95% CI = 4.12-35.04,  $P < 0.001$ ), respectively. This result indicated that the male carrying two copies of APOE- $\epsilon 4$  alleles might have a high risk to develop AD and the female carrying  $\epsilon 3/\epsilon 4$  genotype had a high risk ( $P = 0.017$ ) to develop AD. There was no difference between AD and control groups with respect to  $\epsilon 2/\epsilon 3$  genotype,  $\epsilon 2$

**TABLE 5** Comparison of MRI imaging change between non- $\epsilon 4$  and  $\epsilon 4$  allele groups in non-AD subjects

	$\epsilon 4$ (n = 438)	non- $\epsilon 4$ (n = 2169)	P value
Age (y)	68.5 ± 15.0	70.0 ± 14.8	0.059
Sex (M/F)	267/171	1263/906	0.290
Cerebral infarction	175 (40.0%)	832 (38.4%)	0.532
Age (y)	74.2 ± 13.3	75.9 ± 12.6	0.114
Sex (M/F)	117/58	561/271	0.884
Brain atrophy	24 (5.5%)	105 (4.8%)	0.574
Age (y)	77.8 ± 11.5	73.7 ± 9.6	0.075
Sex (M/F)	18/6	58/47	0.076
Cerebral infarction with brain atrophy	20 (4.6%)	64 (3.0%)	0.081
Age (y)	78.6 ± 11.7	74.6 ± 10.4	0.155
Sex (M/F)	15/5	40/24	0.305
Severe brain atrophy	0	1 (0.05%)	0.832
Age (y)	0	90	—
Sex (M/F)	0	1/0	—

**FIGURE 1** The representative MR imaging photography of Alzheimer's disease patient with and without APOE- $\epsilon$ 4 allele. A and B, The representative MRI image of AD patients carrying APOE- $\epsilon$ 4 allele. C, The representative MRI image of AD patients who were not carrying APOE- $\epsilon$ 4 allele; D, The representative MRI image of non-AD patient



**TABLE 6** Comparison of incidence of cerebral infarction between AD and control groups

	Cases	Cerebral infarction (%)	$\chi^2$	P
AD group	226	154 (68.1)	49.091	<0.001*
Control group	2607	1026 (39.4)		

\*Significant difference

**TABLE 7** Comparison of incidence of cerebral infarction between AD (n = 226) and control groups (n = 274)

	Cases	Cerebral infarction (%)	$\chi^2$	P
AD group	226	154 (68.1)	47.375	<0.001*
Control group	274	102 (37.2)		

\*Significant difference

allele either in female ( $\epsilon$ 2/ $\epsilon$ 3 with  $P = 0.929$ ,  $\epsilon$ 2 with  $P = 0.586$ ) or in male ( $\epsilon$ 2/ $\epsilon$ 3 with  $P = 0.283$ ,  $\epsilon$ 2 with  $P = 0.317$ ), or the entire population ( $\epsilon$ 2/ $\epsilon$ 3 with  $P = 0.372$ ,  $\epsilon$ 2 with  $P = 0.26$ ). These results suggested that the APOE- $\epsilon$ 4 allele might increase the risk for AD in southern China population and such risk was different between the male and female.

## 4 | DISCUSSION

Although the frequencies of APOE genotype in different ethnic populations have been reported, in this study, we reported that the allele frequencies of  $\epsilon$ 2,  $\epsilon$ 3, and  $\epsilon$ 4 alleles were 8.0%, 82.9%, and 9.1%, respectively, among a large population in southern China. This case was similar to the frequencies in the east of China (8.6%, 80.4%, 11%)<sup>22</sup> and in north of China (5.3%, 88.3%, 8.4%).<sup>23</sup> The gene frequencies of APOE- $\epsilon$ 4 are various among different ethnic populations. In worldwide population, the  $\epsilon$ 4 allele tends to be higher in northern Europe such as Iceland, Finland, and Norway (15.7%-18.4%) and lower in Middle East such as Iran (6.1%), Turkey (6.7%), and southern Europe such as Greece (7%) and Italy (5.2%).<sup>11</sup> Previous studies reported that the frequency of APOE- $\epsilon$ 4 allele was about 10% in Asian countries. Our results provide an evidence that the frequency of APOE- $\epsilon$ 4 allele in southern China is in accordance with that of the other regions of China or the other Asian countries. This emphasizes the importance of geographical location and ethnical background of the subjects in the study of APOE genotypes.

The actual cause of AD is not well known, and around 70% of the risk are thought to be genetic. The  $\epsilon$ 4 allele of the APOE is considered as the most common inherited genetic risk for AD.<sup>17</sup> In this work, we found that the frequency of APOE- $\epsilon$ 4 allele in the AD patients was significantly higher than that in the control group. The OR for individual with APOE  $\epsilon$ 4/ $\epsilon$ 4 genotype was 7.64 times higher than that with  $\epsilon$ 3/ $\epsilon$ 3 genotype, whereas the case in people with genotypes

ORs (95% CI)	Female	Male	All
ApoE genotypes			
$\epsilon 3/\epsilon 3$	Referent group	Referent group	Referent group
$\epsilon 2/\epsilon 3$	0.97 (0.48-1.94)	0.72 (0.39-1.31)	0.81 (0.51-1.28)
	$P = 0.929$	$P = 0.283$	$P = 0.372$
$\epsilon 3/\epsilon 4$	1.88 (0.12-3.16)	1.22 (0.76-1.95)	1.47 (1.03-2.08)
	$P = 0.017$	$P = 0.418$	$P = 0.031$
$\epsilon 4/\epsilon 4$	2.16 (0.26-18.27)	12.01 (4.12-35.04)	7.64 (3.11-18.72)
	$P = 0.478$	$P < 0.001$	$P < 0.001$
$\epsilon 3/\epsilon 4 + \epsilon 4/\epsilon 4$	1.89 (1.13-3.15)	1.53 (0.99-2.36)	1.67 (1.20-2.32)
	$P = 0.015$	$P = 0.056$	$P = 0.002$
ApoE allele			
$\epsilon 3$	Referent group	Referent group	Referent group
$\epsilon 2$	0.84 (0.45-1.58)	0.77 (0.46-1.28)	0.80 (0.54-1.18)
	$P = 0.586$	$P = 0.317$	$P = 0.26$
$\epsilon 4$	1.71 (1.09-2.69)	1.76 (1.23-2.52)	1.74 (1.31-2.31)
	$P = 0.019$	$P = 0.002$	$P < 0.001$

**TABLE 8** Odds ratio of ApoE genotypes and alleles with respect to  $\epsilon 3/\epsilon 3$  in the female and male of AD, control groups, and whole population

$\epsilon 2/\epsilon 3$  was only 0.81 times. The risk of  $\epsilon 4$  allele observed in our study was lower than that reported in Japanese and Caucasian, where the people carrying ApoE- $\epsilon 4$  gene are found to have 10-30 times higher risk to develop AD than those who have no ApoE- $\epsilon 4$ .<sup>24</sup> Furthermore, we found that the male carrying two copies of APOE- $\epsilon 4$  alleles had higher risk to develop AD than the case in the female. These results demonstrate that the risk for APOE- $\epsilon 4$  carriers to develop AD is different between the male and female. Overall, our study confirms a strong correlation between ApoE- $\epsilon 4$  and AD in Chinese population, which was different from the cases in Hispanic and African American populations.<sup>25</sup> Moreover, it is notable that not all patients with AD carry an APOE- $\epsilon 4$  allele, and not all carriers of the APOE- $\epsilon 4$  allele will develop AD. This suggests that the APOE- $\epsilon 4$  allele is neither necessary nor sufficient for developing AD.

The underlying mechanism of ApoE- $\epsilon 4$  affecting AD progress has not been fully understood. The pathological change of AD includes the structural atrophy, pathological amyloid depositions, and metabolic alterations in the brain. Indeed, a consistent relationship was found between APOE- $\epsilon 4$  genotype and elevated amyloid burden.<sup>26</sup> To date, several studies have evaluated the relationship between APOE polymorphism and MRI-defined intracranial lesions.<sup>27,28</sup> For example, Wang QY reported that APOE- $\epsilon 4$  increased the burden of cerebrovascular disease. A Chinese study pointed out that APOE- $\epsilon 4$  allele was associated with an increased risk of developing cerebral infarction.<sup>20</sup> Yin et al<sup>29</sup> reported that ApoE- $\epsilon 4$  played an important role in brain atrophy and memory impairment by modulating amyloid production and deposition. Others also observed the effect of APOE- $\epsilon 4$  on gray matter loss in right hippocampus.<sup>30,31</sup> Here, we evaluated the effect of APOE- $\epsilon 4$  allele on the MRI-defined cerebral infarction and brain atrophy. The APOE- $\epsilon 4$  carriers showed an increased brain atrophy in hippocampus and rostral amygdala, compared to non-carriers in AD

patients. Although cognitively normal APOE- $\epsilon 4$  carriers showed no significant increase in the incidence of brain atrophy and cerebral infarction, they have higher incidence for developing brain atrophy in the entire study population. Furthermore, the incidence of cerebral infarction showed no difference between APOE- $\epsilon 4$  carriers and non- $\epsilon 4$  carriers, which is much different from the previous report.<sup>20</sup> Our results suggest that the presence of  $\epsilon 4$  allele increases the incidence of brain atrophy, but had no effect on cerebral infarction. There is no significant evidence that AD pathology is present in the brain many years before the clinical manifestation of the disease, such as amyloid plaques, cerebral infarction, and gray matter atrophy.<sup>1,2</sup> When considering hippocampal atrophy as a baseline AD status, the association of APOE- $\epsilon 4$  with an increased incidence of brain atrophy found in our study could partly explain the reason why APOE- $\epsilon 4$  contributes to AD.

In summary, the present study provides an evidence that APOE- $\epsilon 4$  is linked to increased rates of AD in southern China and the presence of APOE- $\epsilon 4$  has higher incidence of brain atrophy. Our results suggest that genetic factors APOE may increase vulnerability in the gray matter loss of brain in AD patients. These results also provide an evidence that the APOE- $\epsilon 4$  might be recognized as a genomic target for the prevention of AD and brain atrophy. A limitation of this study was that the AD patients were mostly older than 65 years of age and may have some impact on the MRI change. Since some MRI-defined intracranial lesions start at middle age, the effect of APOE- $\epsilon 4$  on neurodegenerative biomarkers may be more prominent if both middle-aged adults and older adults are included.

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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