Apolipoprotein E gene polymorphism and Alzheimer’s disease in Chinese population: a meta-analysis

Mengying Liu1,2, Chen Bian1, Jiqiang Zhang1 & Feng Wen3

1Department of Neurobiology, Chongqing Key Laboratory of Neurobiology, Third Military Medical University, Chongqing 400038, China, 2Student Camp Seven, Third Military Medical University, Chongqing 400038, China, 3Systems Biology Laboratory, Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762, USA.

The relationship between Apolipoprotein E (ApoE) genotype and the risk of Alzheimer’s disease (AD) is relatively well established in Caucasians, but less established in other ethnicities. To examine the association between ApoE polymorphism and the onset of AD in Chinese population, we searched the commonly used electronic databases between January 2000 and November 2013 for relevant studies. Total 20 studies, including 1576 cases and 1741 controls, were retrieved. The results showed statistically significant positive association between risk factor e4 allele carriers and AD in Chinese population (OR = 3.93, 95% CI = 3.37–4.58, P < 0.00001). Genotype ApoE e4/e4 and e4/e3 have statistically significant association with AD as well (e4/e4: OR = 11.76, 95% CI = 6.38–21.47, P < 0.00001; e4/e3: OR = 3.08, 95% CI = 2.57–3.69, P < 0.00001). Furthermore, the frequency of the ApoE e3 is lower in AD than that in the health controls, and the difference of e3 allele is also statistically significant (OR = 0.42, 95% CI = 0.37–0.47, P < 0.00001). No significant heterogeneity was observed among all studies. This meta-analysis suggests that the subject with at least one ApoE e4 allele has higher risk suffering from AD than controls in Chinese population. The results also provide a support for the protection effect of ApoE e3 allele in developing AD.

Although numerous studies have demonstrated the association, inconsistency was presented for different allele frequencies among study populations, particularly in different ethnic and geographical groups. The purpose of conducting this meta-analysis is to reduce heterogeneity and summarize the published evidence on the prevalence of the ApoE polymorphism among patients diagnosed with AD in Chinese population.
Results

Study selection and characteristics. The electronic database search identified 312 references. Of those, 218 records excluded after title review and 103 articles were judged potentially relevant. Following abstracts screened for relevance, 48 full-text articles comprehensively assessed against inclusion criteria. Overall, the initial search with the keywords and the subject terms identified 20 publications, including 1576 cases and 1741 controls that met the inclusion criteria and were eligible for review. Figure 1 shows the study flow.

Of the 20 reports focusing on the relationship between ApoE polymorphism and AD, 18 were from mainland17–34, two were from Taiwan35,36. The distribution of the genotypes in the control group was consistent with Hardy-Weinberg equilibrium (HWE). The detailed characteristics of the included studies were shown in Table 1. The distributions of genotypes in the individual studies were presented in Table 2.

Association between ApoE allele and AD. The results of each allele and genotypes of ApoE in this meta-analysis were listed in Table 3. The heterogeneity between studies was not significant excepting the allele e2. The fixed effect model or the random effect model was employed for calculating the pooled OR. Overall, this meta-analysis showed that the frequency of ApoE e4 allele is higher in AD than that in the health controls, and demonstrated statistically significant positive association between risk factor e4 allele carriers and AD in Chinese population (OR = 3.93, 95% CI = 3.37–4.58, P < 0.00001), as shown in Figure 2. The frequency of the ApoE e3 is lower in AD than that in the health controls and the difference is also

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Table 1 | Main characteristics of the eligible studies

| First author-published year | Geographical location | Total no. of | diagnostic criteria | Mean-age | Cases | Controls |
|-----------------------------|-----------------------|-------------|---------------------|----------|-------|----------|
| Zhu-2000 Shandong           | 36 36                 | NINCDS-ADRA | -                   | -43.42   | -     | -        |
| Huang-2001 Guangzhou        | 41 85                 | DSM-III-R   | 80.8                | 59.6     |
| Jia-2002 Beijing            | 58 60                 | NINCDS-ADRA | 68.6 ± 7.6          | 65.9 ± 8.5 |
| Huang H-2002 Taiwan         | 99 96                 | NINCDS-ADRA | 76.3 ± 6.9          | 72.2 ± 6.9 |
| Bi-2002 Haerbin             | 42 40                 | NINCDS-ADRA | 70.4 ± 6.6          | 68.1 ± 4.3 |
| Zhou-2003 Wulumuqi          | 51 52                 | NINCDS-ADRA | 74.1 ± 9.7          | 69.4 ± 11.4 |
| Chen-2003 Beijing/Shanxi    | 160 195               | NINCDS-ADRA | 69.4 ± 9.5          | 69.8 ± 7.8 |
| Zhang-2004 Shandong         | 32 40                 | CCMD-2-R    | 74.1 ± 7.3          | 63.9 ± 7.3 |
| He-2005 Beijing             | 27 67                 | NINCDS-ADRA | 82.9 ± 7.9          | -        |
| Wang-2006 Taiwan            | 151 161               | DSM-IV      | 74.8 ± 7.9          | 62.5 ± 8.7 |
| Li-2006 Guizhou             | 30 30                 | DSM-IV      | 70.2 ± 11.6         | 71.4 ± 10.5 |
| Yang J-2008 Yunnan          | 58 96                 | NINCDS-ADRA | 74.0 ± 8.48         | 74.2 ± 4.72 |
| Yang L-2008 Henan           | 102 98                | NINCDS-ADRA | 78.5 ± 7.3          | 76.5 ± 9.3 |
| Wu-2009 Shanghai            | 262 118               | NINCDS-ADRA | 76.91 ± 5.10        | 60.72 ± 4.88 |
| Duan-2009 Henan             | 32 76                 | DSM-IV-R    | 70.6 ± 9.8          | 63.5 ± 6.7 |
| Mai-2010 Guangdong          | 88 97                 | DSM-IV-R    | 79.6 ± 9.2          | 79.7 ± 8.6 |
| Jiang-2010 Guanshi          | 79 156                | NINCDS-ADRA | 72.8 ± 9.5          | 71.2 ± 9.3 |
| Zhou C-2012 Chongqing       | 68 72                 | DSM-IV-TR   | 70.6 ± 6.8          | 71.2 ± 6.6 |
| Lv-2012 Shanghai            | 100 106               | NINCDS-ADRA | 77.0 ± 4.8          | 79.5 ± 5.0 |
| Dong-2013 Liaoning          | 60 60                 | NINCDS-ADRA | -                   | -        |

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Association between ApoE genotype and AD. As shown in table 3, the heterogeneity between studies was not significant (I² < 50%) and the fixed effect model was used for calculating the pooled OR. ApoE e4/e4 and ApoE e4/e3 have statistically significant association with AD (e4/e4: OR = 11.76, 95% CI = 6.38–21.47, P < 0.00001; e4/e3: OR = 3.08, 95% CI = 2.57–3.69, P < 0.00001). Figure 3 showed the association between e4/e4 and AD in Chinese population. Genotype e3/e3 also has significant association with AD (OR = 0.39, 95% CI = 0.33–0.45, P < 0.00001). ApoE e3/e2 and ApoE e4/e2 have slight association with AD. There is no association between e2/e2 and AD.

Sensitivity analysis and publication bias. For this meta-analysis, the influence of a single study on the overall meta-analysis estimate was investigated by omitting one study at a time, respectively. The risk ratio was not significantly influenced by omitting any single study.

The distribution of the ORs from individual studies in relation to their respective standard deviation in funnel plot, as shown in Figure 4 and Figure 5. The funnel revealed no evidence of asymmetry. Thus, there was no possibility of publication bias risk in the meta-analysis.

Discussion
ApoE gene, known to mediate the regulation of cholesterol and triglyceride metabolism, is immunochemically localized to the senile plaques, vascular amyloid, and neurofibrillary tangles of AD. In 1993, Strittmatter et al. for the first time demonstrated that there was a highly significant association of ApoE e4 allele and late-onset familial AD. Subsequently, most studies reported gene frequency of ApoE e4 was significantly increased in sporadic AD than in controls. Sando et al. proved that ApoE e4 is a very strong risk factor for AD in the population of central Norway, and lowers age at onset of late onset AD (LOAD) significantly. Rhinn et al. identified an ApoE e4 associated molecular pathway that promotes LOAD; Genin et al. demonstrated that ApoE e4 is a risk factor not only for late-onset but for early-onset AD as well. Together, these results urge a reappraisal of the impact of ApoE in AD.

Previous meta-analysis demonstrated that ApoE e4 genotype prevalence varies among AD patients by region and within each country: the highest estimates were in Northern Europe; the lowest estimates were in Asia and Southern Europe. To further explore and examine the association of prevalence district of ApoE genotype and AD risk, we conducted this meta-analysis only in Chinese population. Overall, our results showed that the risk of developing AD in e4 allele carriers was 3.93-fold higher than individuals without e4 allele. The risk of developing AD in individuals with e4/e4 genotype was 11.76-fold higher than individuals without e4/e4 genotype. There probably exists a dose-dependent association between the number of ApoE e4/e4 allele and the risk of AD in Chinese population. Furthermore, the frequency of the ApoE e3 is lower in AD than that in the health controls and the difference is also statistically significant, implying the protection effect of e3 allele in developing AD. No association was found between e2 allele and AD risk.

The e4 allele of ApoE is the “risk” variant for several phenotypes compared with e3 (“neutral”), and e2 (generally considered “protective”, although less consistently). The ApoE gene confers differential susceptibility to AD etiology depending on the combination of the 3 alleles as well as the age and ethnicity of the person. The e4 allele of ApoE is the strongest genetic risk factor for the development of AD. Although multiple genetic and environmental risk factors are involved in LOAD pathogenesis, overall impairment in Aβ clearance is probably a major contributor to disease development. Accumulation of amyloid-β (Aβ) is hypothesized to initiate synaptic and neuronal dysfunction that ultimately lead to neuronal cell death in AD, and several lines of evidence strongly suggest that the differential effects of ApoE isoforms on Aβ aggregation and/or clearance plays a major role in AD pathogenesis. The ApoE isoforms could influence the risk for AD via other mechanisms as well. Relative to the common e3 allele, possession of e4 increases disease risk and decreases age at onset in a dose-dependent manner. In contrast, possession of the e2 allele may confer protection against AD, as carriers of this allele are less likely to develop the disease than e3 homozygotes. The ApoE e4 allele is associated with greater accumulation of both Aβ plaques and neurofibrillary tangles than the e3 allele, while carriers of the e2 allele typically develop less AD-related pathology than both e4 carriers and e3 homozygotes. Pomara et al.

Table 2 | Distribution of genotypes in the individual studies

| First author | e4/4 | e3/e4 | e3/e3 | e2/e4 | e2/e3 | e2/e2 | e4/2 | e3/2 | e2/2 | e4/3 | e3/3 | e2/3 | e2/2 |
|--------------|------|-------|-------|-------|-------|-------|------|------|------|------|------|------|------|
| Zhu 2000     | 3    | 18    | 12    | 5     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Huang 2001   | 4    | 20    | 13    | 3     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Jia 2002     | 1    | 29    | 15    | 2     | 0     | 0     | 1    | 0    | 0    | 0    | 0    | 0    | 0    |
| Huang 2002   | 6    | 53    | 23    | 9     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Bi 2002      | 2    | 20    | 13    | 2     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Zhou 2003    | 1    | 26    | 19    | 2     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Chen 2003    | 1    | 85    | 47    | 1     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Zhang 2004   | 1    | 18    | 9     | 2     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| He 2005      | 0    | 13    | 9     | 4     | 1     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Wang 2006    | 3    | 75    | 54    | 7     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Li 2006      | 2    | 17    | 9     | 1     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Yang L 2008  | 10   | 44    | 35    | 5     | 1     | 0     | 1    | 0    | 0    | 0    | 0    | 0    | 0    |
| Yang J 2008  | 1    | 40    | 13    | 4     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Wu 2009      | 30   | 118   | 102   | 12    | 1     | 1     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Duan 2009    | 5    | 8     | 12    | 4     | 0     | 0     | 1    | 0    | 0    | 0    | 0    | 0    | 0    |
| Mai 2010     | 6    | 45    | 18    | 14    | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Jiang 2010   | 2    | 17    | 20    | 6     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Zhou C 2012  | 5    | 8     | 17    | 2     | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Lv 2012      | 11   | 44    | 33    | 10    | 0     | 0     | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| Dong 2013    | 4    | 27    | 23    | 3     | 0     | 0     | 1    | 0    | 0    | 0    | 0    | 0    | 0    |

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showed a potential effect of the ApoE ε2 allele and of family history of Alzheimer’s disease on brain amyloid-β in normal elderly51. Hostage et al. confirmed and extended prior data on the opposing effects of the ApoE ε4 and ε2 alleles on hippocampal morphology across the spectrum of cognitive aging48. This general pattern holds for many physiological phenomena influenced by ApoE genotype, and potentially related to AD, including measures of synaptic plasticity and repair46, antioxidant properties, and certain immune responses49, and cholesterol levels50.

Currently, genetic testing of ApoE ε4 carrier status is not routinely considered in clinical practice. As ApoE genotype determines AD risk, and ApoE has crucial roles in cognition55,56, ApoE might offer an attractive alternative target for AD therapy. ApoE genotype status could be included in clinical trial enrolment criteria, as some therapies might be effective only in specific ApoE genotypes. In addition, ApoE is a crucial regulator of the innate immune system, with ApoE ε4 promoting pro-inflammatory responses that could exacerbate AD pathogenesis.

Several limitations were presented in this meta-analysis. Firstly, in AD group of retrieved case-control studies, there may exist mixed dementia. It might increase the apparent association of ApoE with AD, since ApoE ε4 allele is associated with dementia. Secondly, ApoE polymorphism may interact with other known and unknown risk factors which should be considered. Thirdly, the selected studies may have more subject to bias and artifact than prospective studies.

In conclusion, our meta-analysis suggests that ApoE ε4 carrier is associated with AD and provide a support for the protection effect of ApoE ε3 allele in developing AD in Chinese population. Although ApoE polymorphism is a well-studied genetic risk factor for developing AD, in some regions most patients do not carry this genotype.

### Table 3 | Meta-analysis of Apolipoprotein E gene polymorphism in Alzheimer’s disease

| Allele | Fix-effect model | Random-effect model |
|--------|------------------|---------------------|
|        | OR (95% CI)      | P       | Phet | I²   | OR (95% CI) | P       | Phet | I²   |
| ApoE ε2 | 0.88 (0.73, 1.07) | 0.20    | 0.002 | 61%  | 0.93 (0.66, 1.29) | 0.65    | 0.0002 | 61%  |
| ApoE ε3 | 0.42 (0.37, 0.47) | <0.00001 | 0.07 | 34%  | 0.42 (0.36, 0.49) | <0.00001 | 0.07 | 34%  |
| ApoE ε4 | 3.93 (3.37, 4.58) | 0.81    | 0.001 | 0%   | 3.89 (3.33, 4.54) | <0.00001 | 0.07 | 0%   |

| Genotype | Fix-effect model | Random-effect model |
|----------|------------------|---------------------|
|          | OR (95% CI)      | P       | Phet | I²   | OR (95% CI) | P       | Phet | I²   |
| ApoE ε4/ε4 | 11.17 (6.38, 21.47) | <0.00001 | 0.98 | 0%   | 9.84 (5.28, 18.34) | <0.00001 | 0.98 | 0%   |
| ApoE ε3/ε3 | 0.39 (0.33, 0.45) | <0.00001 | 0.25 | 16%  | 0.38 (0.33, 0.45) | <0.00001 | 0.25 | 16%  |
| ApoE ε2/ε2 | 1.06 (0.55, 2.00) | 0.86    | 0.60 | 0%   | 1.09 (0.52, 2.27) | 0.83    | 0.60 | 0%   |
| ApoE ε3/ε2 | 3.08 (2.57, 3.69) | <0.00001 | 0.06 | 35%  | 3.16 (2.48, 4.02) | <0.00001 | 0.06 | 35%  |
| ApoE ε4/ε2 | 0.67 (0.53, 0.85) | <0.0008 | 0.18 | 23%  | 0.66 (0.49, 0.88) | 0.005    | 0.18 | 23%  |
| ApoE ε4/ε2 | 2.10 (1.35, 3.26) | 0.001   | 0.32 | 11%  | 1.92 (1.13, 3.28) | 0.02    | 0.32 | 11%  |

ApoE ε4 allele carriers and AD in Chinese population.

Figure 2 | Forest plot on the association between ε4 allele carriers and AD in Chinese population.
Therefore, additional research with well-designed and large sample sizes is needed to be able to understand both other genetic and environmental risk factors in the future.

Methods

Identification and eligibility of relevant studies. We conducted a comprehensive literature search using the electronic database of PubMed, Wanfang database and CNKI (China National Knowledge Infrastructure) for relevant articles assessing the association of ApoE polymorphism and AD in Chinese population from January 2000 to November 2013. The Medical Subject Heading (MeSH) terms “Alzheimer’s disease”, “AD”, “ApoE”, “Apolipoprotein E”, and “polymorphism” were employed as the searching words. The equivalent Chinese terms were used in the Chinese databases. All studies matching the eligibility criteria were retrieved, and references were checked for other relevant publications.

Criteria for article screening. Studies eligible for inclusion in this meta-analysis must meet the following criteria: 1) case-control or cohort study; 2) measure the relationship between ApoE polymorphism and AD; 3) clinical diagnosis of AD based on standards of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer’s Disease and Related Disorders Association (NINCDS/ADRDA) work group; 4) the results were expressed as odds ratio (OR) and corresponding 95 percent confidence interval (95% CI); 5) genotype distribution of control for a certain polymorphism must be in HWE; and 6) when the same authors reported two or more publications on possibly the same patient populations, only the most recent or complete study was included into this meta-analysis.

Figure 3 | Forest plot on the association between ε4/ε4 genotype and AD in Chinese population.

Figure 4 | Funnel plot on the association between ε4 allele carriers and AD.
Cochran's Q test and I² statistics. P-value less than 0.05 and I² less than 50% were considered significant. The heterogeneity for the included articles was evaluated using the pooled OR was determined by the Z test, and a P value less than 0.05 was used to assess the relationship of ApoE polymorphism and AD. The significance of SCIENTIFIC REPORTS

Statistical analysis

discussing with the third expert. Data retrieved from the reports included first author, publication year, demographics, number of cases and controls, distribution of genotypes, and the diagnosis criteria of AD.

Genotypes, and the diagnosis criteria of AD.

Two investigators independently extracted data and reached a consensus on all of the items. Any disagreement was resolved by

Statistical analysis. The odd ratio (OR) with 95% confidence intervals (95% CI) was calculated by the Z test, and a P value less than 0.05 was considered significant. When there was no significant heterogeneity across the included studies ($I^2 < 50\%$), the fixed effect model was used; When there was significant heterogeneity across the included studies ($I^2 \geq 50\%$), the random effect model was used\(^5\). To assess whether our results were substantially influenced by the presence of any individual study, we conducted a sensitivity analysis by systematically removing each study and recalculating the significance of the result. Begg's funnel plot was performed to examine the publication bias. Analyses were carried out using the Review manager 5.2 (The Cochrane Collaboration). All tests were two sided.

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Figure 5 | Funnel plot on the association between ε4/ε4 genotype and AD.
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