Current Medical Science
DOI https://doi.org/10.1007/s11596-019-2007-5 39(1):111-117,2019

Apolipoprotein E Gene Polymorphisms Are Risk Factors for Spontaneous Intracerebral Hemorrhage: A Systematic Review and Meta-analysis*

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Summary: Intracerebral hemorrhage (ICH) is a serious clinical disease with high morbidity, whose pathogenesis might be related to apolipoprotein E (APOE) gene polymorphisms. To comprehensively evaluate the risk factors for ICH occurrence, we performed a meta-analysis. We searched online databases to identify eligible studies based on the relationship between APOE genetic polymorphisms and ICH occurrence risk. Specific and pooled odds ratios (ORs) were calculated and by assessing small study bias, we drew the relationship between APOE polymorphisms and ICH risk. We included 15 eligible studies in our study containing a total of 1642 ICH samples and 5545 normal controls. The comparison of ε4 and ε3 APOE genotypes revealed that specific and pooled ORs showed a significantly increased odds ratio in ICH patients with the ε4 genotype, indicating that ε4 gene is a risk factor for ICH occurrence, and the heterogeneity is acceptable. Similarly, it was found that the ε2 genotype also contributed to the incidence rate of ICH. However, after the subgroup analysis by ethnicity, this APOE genetic polymorphism acted as a harmful factor only in white populations, but did not show an effect in Asian populations. It was suggested that both ε2 and ε4 APOE alleles were risk factors for ICH in general. They were risk factors in white populations only, neither had a detectable effect in Asian populations after subgroup analysing by ethnicity.

Key words: intracerebral hemorrhage; apolipoprotein E; allele; genetic polymorphism; meta-analysis

Intracerebral hemorrhage (ICH) is a serious clinical disease, characterized by high lethality and disability. ICH is the most common cause of hemorrhagic stroke, and the annual incidence rate is twice that of subarachnoid hemorrhage. ICH has also been reported to have a higher fatality rate than the ischemic type[1]. ICH pathogenesis has been reported to be related to many factors, such as older age, ethnicity, hypertension, lower low-density lipoprotein cholesterol (LDL-C), and lower triglyceride levels[2].

The apolipoprotein E (APOE) gene is located on chromosome 19, and encodes a protein composed of 299 amino acids. The APOE protein has structural polymorphisms with three different alleles, ε2, ε3 and ε4, producing three different protein products, E2, E3 and E4. ε3 has the highest gene frequency in human population, therefore ε2 and ε4 are referred to as mutant types. It has been shown that APOE plays a role in lipid metabolism and transport, tissue injury repair, peripheral nerve injury and regeneration, and immunoregulation and modulation of cell growth and differentiation[3]. APOE has also been reported to contribute to the behavior of β-amyloid which is the component of artery atheromatous plaques[4, 5].

Several studies have reported that APOE gene polymorphisms are related to ICH occurrence but the conclusions remain controversial. A meta-analysis published in 2014 showed that the APOE ε4 allele is associated with a higher risk of ICH, while the ε2 allele did not show a statistical difference. We therefore collected data from all relevant studies and performed a meta-analysis. Compared with previous reports, we

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*This study was supported by the National Natural Science Foundation of China (No. 81471201 and No. 81171089).

have enrolled more studies, more cases, and optimized the study methods, and found different conclusions. This study provides some new insights into the pathogenesis of ICH.

1 MATERIALS AND METHODS

1.1 Study Identification

We searched PubMed and Embase (1980 to April 2017) using the following search terms: “APOE” “apolipoprotein E” “hemorrhagic stroke” “brain hemorrhage” “intracerebral hemorrhage” “intracranial hemorrhage” also with a combination of MeSH (medical subject headings) terms. Two researchers worked independently on these searches and also checked the mentioned supplementary articles of all relevant studies and reviews, and searched for them. Eligible studies were reviewed in duplicate to determine whether or not they should be included in the meta-analysis. If necessary, we attempted to contact the principal investigators of the retrieved articles to acquire additional data.

1.2 Inclusion/Exclusion Criteria

The inclusion criteria were as follows: spontaneous cerebral hemorrhage; original studies for humans and detailed data to extract APOE allele frequency. Exclusion criteria were as follows: non-spontaneous cerebral hemorrhage including tumor, vascular malformation, trauma, drug-related cerebral hemorrhage and other kind of hemorrhage; studies that did not have normal control groups; studies that did not provide detailed gene allele frequencies; studies that could not trace original data and duplicate reports.

1.3 Data Collection

Two authors extracted items independently, including general study information (first author, publication date), data collection cycle, study design methods, population characteristics (ethnic region, age, sex, history of smoking, alcohol use, blood lipid conditions, blood glucose conditions, blood pressure conditions), APOE genotyping methods, hemorrhage area, APOE alleles in cases of ICH and control groups, and study conclusions. From each clinical trial, the researchers extracted, without exception, all available data from reported outcomes available, text, and graphs.

1.4 Methodological Quality of Studies

The quality of each experiment was assessed according to the Newcastle-Ottawa Quality Assessment Scale. Ten points represent the perfect score, the higher the score, the better quality of data the study has. Meanwhile, characteristics described in the studies were also collected in the checklist, which consisted of the following: (1) reporting of a sample size calculation, (2) blind assessment of genotyping outcome, (3) description of population characteristics, including ethnic region, age, sex, history of smoking, alcohol use, blood lipid levels, blood glucose level, blood pressure, (4) description of data collection, including the collecting period and source of samples, (5) descriptions of diagnostic criteria, including CT, MRI and clinical performance.

1.5 Statistical Analysis

STATA SE 12.0 was used for data analysis. The Mantel-Haenszel method for fixed effects and the Der-Simonian-Laird method for random effects were applied to estimate the pooled odds ratio (OR) and 95% confidence interval (CI). I^2, Q test and the corresponding P value were used to estimate the heterogeneity of the studies. The publication bias was assessed using Egger regression. A P value of <0.05 was considered statistically significant.

2 RESULTS

2.1 Study Characteristics

We searched literature databases using the keywords (APOE OR apolipoprotein E) AND (intracerebral hemorrhage OR ICH OR hemorrhagic stroke OR brain hemorrhage) with a combination of MeSH terms. This identified 300 results from PubMed and 84 results from Embase. After filtering all the articles, 15 eligible studies[6–20] were enrolled into our study (fig. 1), containing a total of 1642 ICH samples and 5545 normal controls (table 1). Populations in the study were from Europe, America, Africa, and Asia.

2.2 Quality Score

Sample size, ICH diagnostic methods, genotyping method, population characteristics, study type, data type, data description as the quality score standard, all the included studies processed with our pre-set criteria, and the scores of 15 studies are displayed in table 2. No study fulfilled all the criteria, but a large number of them had a high-quality score. In the Newcastle-Ottawa Scale, all included studies tended to get high marks (table 2).

2.3 Meta-analysis (Quantitative Synthesis and Heterogeneity Analysis)

Because ethnicity and genetic polymorphisms are vitally interrelated, we performed a subgroup analysis by ethnicity based on the hypothesis that ethnicity-related polymorphisms might play a role in the relationship of APOE allele polymorphisms and ICH occurrence rates. We compared ε4 and ε3 and found that the specific and pooled ORs showed a significantly increased odds ratio in ICH patients with the ε4 genotype (fig. 2). This indicated that the ε4 allele was a risk factor for ICH occurrence, and the heterogeneity was acceptable. The ε4 allele was a risk factor for ICH occurrence in the white population, but not for black or yellow people. As shown in fig. 3, the ε2 allele also contributes to the incidence rate of ICH in general, but

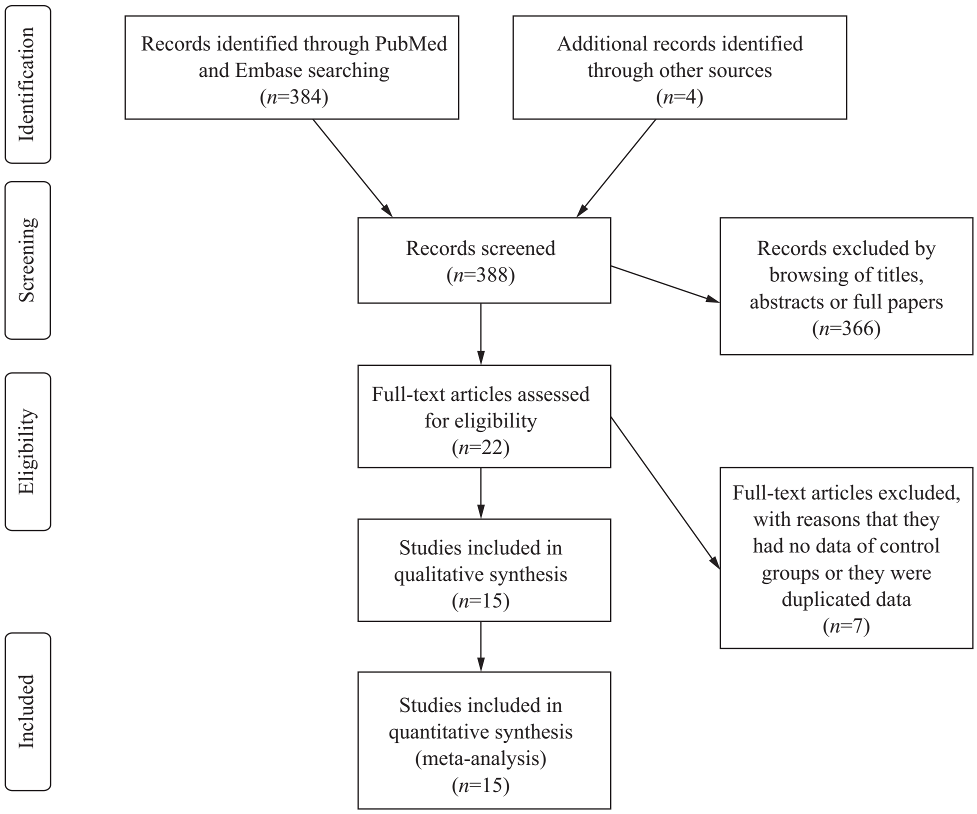

Fig. 1 Flow diagram of preferred reporting items for systematic reviews and meta-analyses (PRISMA)

Table 1 Frequencies of APOE alleles of studies

Studies	ε2 of cases	ε2 of controls	ε3 of cases	ε3 of controls	ε4 of cases	ε4 of controls
Atadzhanov 2013	4	32	19	138	13	62
Boumendjel 2013	3	25	70	429	8	55
Catto 2000	5	44	95	446	20	88
Chen 2009	17	22	179	239	21	19
Chowdhury 2011	2	13	146	333	12	34
Das 2016	32	64	393	1035	75	141
Duzenli 2009	8	29	71	201	3	22
Garcia 1999	2	2	82	86	12	8
Kokubo 2000	14	103	131	1913	23	236
McCarron 1999	20	189	143	1587	41	302
Misra 2013	7	15	183	343	10	18
Seifert 2006	30	43	309	456	47	61
Tasdemir 2007	3	2	60	53	7	5
Woo 2013	328	312	2286	2815	542	525
Zhang 2012	26	26	280	314	54	20

when it comes to racial classification, APOE ε2 alleles, in accordance with ε4 allele, also showed a link to ICH in white people and insignificant relationship with Asian people, however, in black people the sample size was too small to make a conclusion.

We did not find obviously statistical heterogeneity in the progress. All the heterogeneity test results are shown in table 3.

2.4 Publication Bias

We applied Egger's test for examination of publication bias (fig. 4 and fig. 5). According to the test results, comparison between ε4 and ε3 did not show significant publication bias (P=0.753). However, a prominent publication bias for comparison between ε2 and ε3 was identified (P=0.023).

3 DISCUSSION

This meta-analysis of the link between APOE polymorphisms and ICH included 15 studies, 1642 ICH samples and 5545 normal controls. The data suggest the following: (1) Both ε2 and ε4 APOE alleles are

Table 2 Study quality score according to quality score criteria and the Newcastle-Ottawa Scale

Authors	Sample size (0–2)			Genotyping methods (0–1)		Population characteristics (0–2)								Data collection (0–1)		Diagnostic criteria (0–1)		Total points	NOS points
	>200	100–200	<100	Double blind	Undescribed or undouble blind	Age	Gender	Blood glucose	Blood lipids	Blood pressure	Smoking	Alcohol drinking	Hemorrhage location	Period	Population	Described	Undescribed		
Atadzhanov			√		√	√	√	√	√	√	√	√		√	√	√		4	8
Boumendjel			√		√	√	√		√					√	√	√		2	8
Catto		√			√	√	√	√	√	√	√				√	√		3	8
Chen	√				√	√	√	√	√	√	√	√			√	√		5	8
Chowdhury			√		√	√	√	√		√				√	√	√		3	8
Das			√		√	√	√	√	√	√	√	√		√	√	√		4	8
Duzenli			√	√		√	√	√		√	√				√	√		3	8
Garcia-CAA			√	√		√	√						√		√	√		3	9
Kokubo			√		√	√	√	√		√	√	√		√	√	√		4	8
McCarron	√				√	√	√			√					√	√		3	8
Mirsa		√			√	√	√	√	√	√	√	√			√	√		4	8
Seifert		√			√	√	√	√	√	√			√	√	√	√		4	8
Tasdemir			√		√	√	√	√	√	√	√	√	√	√	√	√		4	8
Woo	√				√	√	√		√	√		√	√		√	√		4	9
Zhang		√		√		√	√	√	√	√	√	√	√	√	√	√		6	8

Table 3 Heterogeneity test of APOE alleles and ICH risk

People	ε4 *versus* ε3 group					ε2 *versus* ε3 group				
	Heterogeneity statistic	Degrees of freedom	*P*	*I*-squared	Tau-squared	Heterogeneity statistic	Degrees of freedom	*P*	*I*-squared	Tau-squared
Black	0.00	0	–	–	0.0175	0.00	0	–	–	0.0000
White	2.50	5	0.777	0.0%	0.0000	4.58	5	0.470	0.0%	0.0000
Yellow	15.17	7	0.034	53.8%	0.1055	7.43	7	0.386	5.7%	0.0081
Overall	18.78	14	0.173	25.5%	0.0175	12.23	14	0.588	0.0%	0.0000

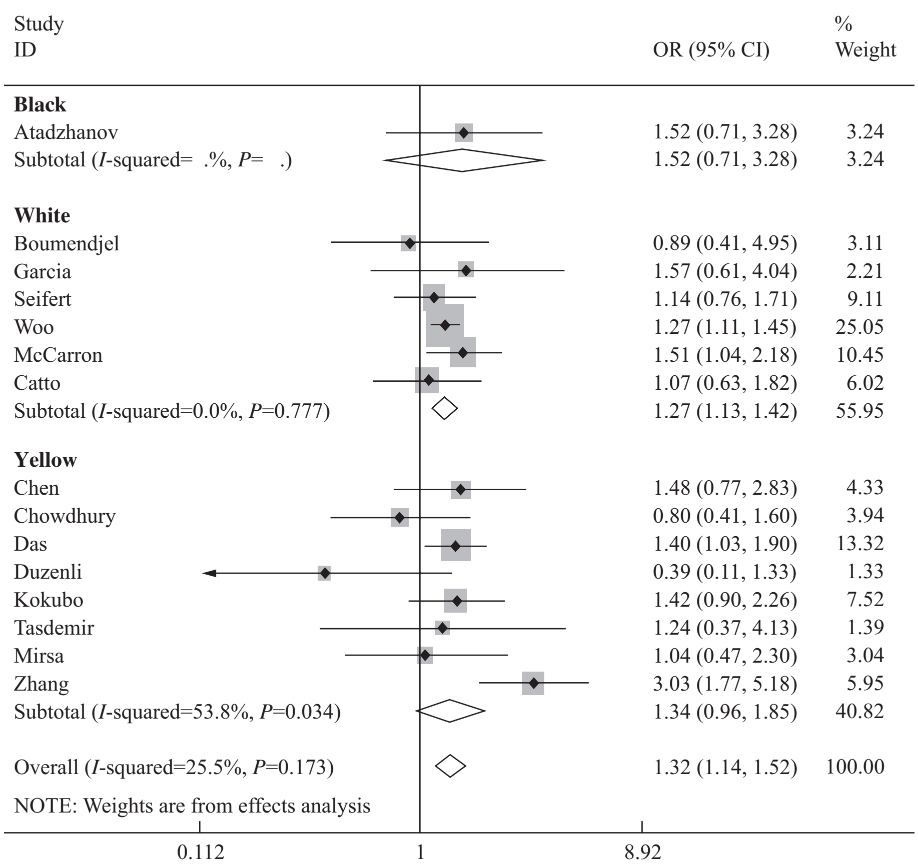

Fig. 2 Forest plot and subgroup analysis of APOE alleles and ICH (ε4 *vs.* ε3)

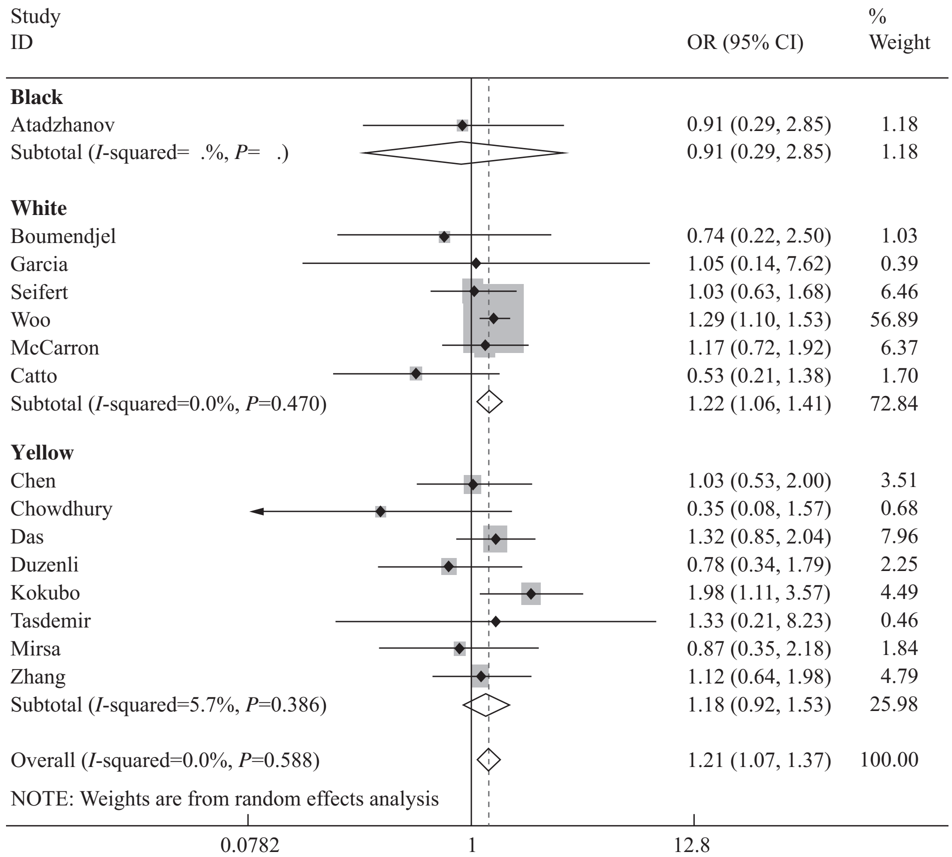

Fig. 3 Forest plot and subgroup analysis of APOE alleles and ICH (ε2 *vs.* ε3)

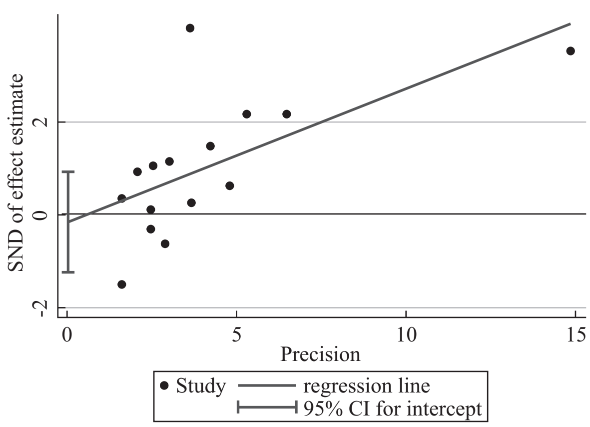

Fig. 4 Egger's test for the publication bias of ε4 *vs.* ε3

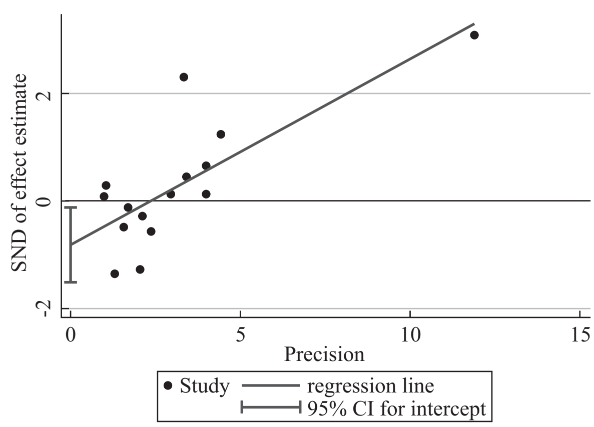

Fig. 5 Egger's test for the publication bias of ε2 *vs.* ε3

risk factors for ICH in general. (2) From the subgroup analysis we found that both ε4 allele and ε2 allele are risk factors in white populations only, neither of them increases ICH occurrence rate in Asian populations. The sample size was too small to draw any clear conclusions for black populations.

APOE has been reported to be associated with many kinds of nervous system diseases. Besides cerebrovascular diseases, Corder *et al* reported that ε4 gene dosage is a major risk factor for late onset Alzheimer disease (AD) and, in these families, homozygosity for ε4 was virtually sufficient to cause AD by the age of 80[21]. It has also been reported that the ε2 allele displayed a protective effect in sporadic AD. The opposing effects of ε2 and ε4 further support the direct involvement of APOE in the pathogenesis of AD[22].

APOE also has an impact on human cognitive function. Bartzokis found that APOE alleles shift the age at onset of cognitive decline[23]. The APOE ε4 allele has a significantly higher frequency among patients with ischemic stroke than the healthy controls, and the authors concluded that APOE ε4 allele is an independent risk factor for ischemic stroke and ischemic coronary heart disease among Greek patients[24].

Besides spontaneous cerebral hemorrhage, APOE has also been reported to be associated with other kinds of cerebral hemorrhage. Falcone *et al* conducted a case-control study and found that APOE genetic diversity was a strong risk factor for warfarin-related intracerebral hemorrhage, especially in the lobar hemorrhage[25]. In another study conducted by Leclercq *et al*, it was found that the risk of cerebral amyloid angiopathy (CAA) for carriers of ε4 was 8.4 times higher than that for the non-ε4 carriers in traumatic brain injury[26].

The mechanism of how APOE affects ICH occurrence rate has not been clearly elucidated yet. Some hypotheses have advocated that APOE ε2 and ε4 can cause abnormalities in lipid metabolism, ε4 by enhancing amyloid deposition and ε2 by causing amyloid-laden vessels to undergo the vasculopathic changes that lead to rupture. Above all, the vessels are more fragile in response to stress[27]. Tola *et al* reported that both truncated APOE and the APOE peptide increase intracellular calcium levels, and cause death of hippocampal neurons in rat embryos[28]. However, there is debate in the field. Lynch *et al* reported that APOE might mediate a protective effect by modulating glial activation and TNFα production, thus decreasing cerebral edema[29].

Studies on the relationship between APOE and ICH are of importance. APOE polymorphisms play not only a significant role in predicting the risk of ICH occurrence, but also relate to its severity. Hematoma after bleeding is considered to be an important indicator of mortality and poor outcome after ICH[30]. Liaquat *et al* found that patients with ε4 APOE alleles tend to have larger hematomas during head injury[31]. Biffi *et al* stated that this relationship can be partly explained by the vessel fragility near the hematoma, where substances released by hematoma could also contribute to vessel leakage[32].

A strong association between the ε4 APOE genotype and a poor neurological outcome after ICH was reported[33]. Biffi *et al* found that APOE ε2 may contribute to blood vessel changes, influencing the severity and clinical course of hemorrhage, increasing the risk of mortality and poor functional outcomes of lobar ICH[32]. As for reccurrence rates, Raffeld *et al* reported that APOE ε4 contributed to ICH recurrence in nonlobar brain regions[34]. It was also partly regulated by lipid metabolism, especially by LDL levels.

Our studies found that both ε2 and ε4 alleles are risk factors for ICH occurrence rates in general. The study size was too small to make any firm conclusions on black people, however, the ε2 allele was a risk factor for white people only, whereas the ε4 allele does not show any racial difference. We might ascribe this result to the following observations. First, insufficient study number might provide incomplete understanding about genetic effects. Second, as reported before, ICH occurrence rates might be associated with hypertension, alcohol abuse, heavy smoking and other factors. The corresponding effect might cause disturbance to our genetic analysis.

Our study has some advantages and limitations. As a limitation, publication bias was examined in the ε2 versus the ε3 group by Egger's test, which suggested the presence of a potential publication bias, a language bias, inflated estimates by a flawed methodologic design in smaller studies, and/or a lack of publication of small trials with opposite results. As for the advantages, in accordance with the previous meta-analysis, we also found the relationship between ε4 and the occurrence risk of ICH. But we have a relatively larger population, which means a more solid conclusion and a smaller statistical bias. We also found a connection between ε2 and ICH morbidity, which is inconsistent with previous reports. This inconsistency might be attributed to inaccuracies in stroke classification, small sample size, different age ranges, or the removal of fatal cases in the acute phase[13].

In conclusion, we find that APOE ε2 and ε4 are both risk factors for the occurrence of spontaneous ICH, and the influence differs with ethnicity. The mechanism remains controversial, and more work needs to be done in future studies.

Conflict of Interest Statement

The authors declare that they have no competing interests.

REFERENCES

1 Brown RD, Whisnant JP, Sicks JD, *et al*. Stroke incidence, prevalence, and survival: secular trends in Rochester, Minnesota, through 1989. Stroke, 1996,27(3):373-380
2 Sturgeon JD, Folsom AR, Longstreth WJ, *et al*. Risk factors for intracerebral hemorrhage in a pooled prospective study. Stroke, 2007,38(10):2718-2725
3 Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science, 1988,240(4852):622-630
4 Hatters DM, Budamagunta MS, Voss JC, *et al*. Modulation of apolipoprotein E structure by domain interaction: differences in lipid-boud and lipid-free forms. J Biol Chem, 2005,280(40): 34 288-34 295
5 Ophir G, Amariglio N, Jacob-Hirsch J, *et al*. Apolipoprotein E4 enhances brain inflammation by modulation of the NF-kappaB signaling cascade. Neurobiol Dis, 2005,20(3):709-718
6 Atadzhanov M, Mwaba MH, Mukomena PN, *et al*. Association of the APOE, MTHFR and ACE Genes Polymorphisms and Stroke in Zambian Patients. Neurol Int, 2013,5(4):e20
7 Boumendjel S, Khodja D, Hamri A, *et al*. Apolipoprotein E polymorphism and cerebral stroke. Ann Biol Clin (Paris), 2013,71(1):21-26
8 Chen YC, Lee-Chen GJ, Wu YR, *et al*. Analyses

of interaction effect between apolipoprotein E polymorphism and alcohol use as well as cholesterol concentrations on spontaneous deep intracerebral hemorrhage in the Taiwan population. Clin Chim Acta, 2009,408(1-2):128-132
9 Chowdhury AH, Yokoyama T, Kokubo Y, *et al*. Apolipoprotein E genetic polymorphism and stroke subtypes in a Bangladeshi hospital-based study. J Epidemiol, 2001,11(3):131-138
10 Das S, Kaul S, Jyothy A, *et al*. Association of APOE (E2, E3 and E4) gene variants and lipid levels in ischemic stroke, its subtypes and hemorrhagic stroke in a South Indian population. Neurosci Lett, 2016,628:136-141
11 Duzenli S, Pirim I, Gepdiremen A, *et al*. Apolipoprotein E polymorphism and stroke in a population from eastern Turkey. J Neurogenet, 2004,18(1):365-375
12 Garcia C, Pinho EMT, Rocha L, *et al*. Cerebral hemorrhage and apoE. J Neurol, 1999,246(9): 830-834
13 Kokubo Y, Chowdhury AH, Date C, *et al*. Age-dependent association of apolipoprotein E genotypes with stroke subtypes in a Japanese rural population. Stroke, 2000,31(6):1299-1306
14 Seifert T, Lechner A, Flooh E, *et al*. Lack of association of lobar intracerebral hemorrhage with apolipoprotein E genotype in an unselected population. Cerebrovasc Dis, 2006,21(4):266-270
15 Tasdemir N, Tamam Y, Toprak R, *et al*. Association of apolipoprotein E genotype and cerebrovascular disease risk factors in a Turkish population. Int J Neurosci, 2008,118(8):1109-1129
16 Woo D, Deka R, Falcone GJ, *et al*. Apolipoprotein E, statins, and risk of intracerebral hemorrhage. Stroke, 2013,44(11):3013-3017
17 Zhang R, Wang X, Liu J, *et al*. Apolipoprotein E gene polymorphism and the risk of intracerebral hemorrhage in the Chinese population. Genet Test Mol Biomarkers, 2012,16(1):63-66
18 Mccarron MO, Hoffmann KL, Delong DM, *et al*. Intracerebral hemorrhage outcome: apolipoprotein E genotype, hematoma, and edema volumes. Neurology, 1999,53(9):2176-2179
19 Catto AJ, Mccormack LJ, Mansfield MW, *et al*. Apolipoprotein E polymorphism in cerebrovascular disease. Acta Neurol Scand, 2000,101(6):399-404
20 Misra UK, Kalita J, Somarajan BI. Recurrent intracerebral hemorrhage in patients with hypertension is associated with APOE gene polymorphism: a preliminary study. J Stroke Cerebrovasc Dis, 2013,22(6):758-763
21 Corder EH, Saunders AM, Strittmatter WJ, *et al*. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science, 1993,261(5123):921-923
22 Corder EH, Saunders AM, Risch NJ, *et al*. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet, 1994,7(2):180-184
23 Bartzokis G, Lu PH, Geschwind DH, *et al*. Apolipoprotein E affects both myelin breakdown and cognition: implications for age-related trajectories of decline into dementia. Biol Psychiatry, 2007,62(12):1380-1387
24 Konialis C, Spengos K, Iliopoulos P, *et al*. The APOE E4 Allele Confers Increased Risk of Ischemic Stroke Among Greek Carriers. Adv Clin Exp Med, 2016,25(3):471-478
25 Falcone GJ, Radmanesh F, Brouwers HB, *et al*. APOE epsilon variants increase risk of warfarin-related intracerebral hemorrhage. Neurology, 2014,83(13):1139-1146
26 Leclercq PD, Murray LS, Smith C, *et al*. Cerebral amyloid angiopathy in traumatic brain injury: association with apolipoprotein E genotype. J Neurol Neurosurg Psychiatry, 2005,76(2):229-233
27 Greenberg SM, Vonsattel JP, Segal AZ, *et al*. Association of apolipoprotein E epsilon2 and vasculopathy in cerebral amyloid angiopathy. Neurology, 1998,50(4):961-965
28 Tolar M, Keller JN, Chan S, *et al*. Truncated apolipoprotein E (ApoE) causes increased intracellular calcium and may mediate ApoE neurotoxicity. J Neurosci, 1999,19(16):7100-7110
29 Lynch JR, Pineda JA, Morgan D, *et al*. Apolipoprotein E affects the central nervous system response to injury and the development of cerebral edema. Ann Neurol, 2002,51(1):113-117
30 Davis SM, Broderick J, Hennerici M, *et al*. Hematoma growth is a determinant of mortality and poor outcome after intracerebral hemorrhage. Neurology, 2006,66(8):1175-1181
31 Liaquat I, Dunn LT, Nicoll JA, *et al*. Effect of apolipoprotein E genotype on hematoma volume after trauma. J Neurosurg, 2002,96(1):90-96
32 Biffi A, Anderson CD, Jagiella JM, *et al*. APOE genotype and extent of bleeding and outcome in lobar intracerebral haemorrhage: a genetic association study. Lancet Neurol, 2011,10(8):702-709
33 Alberts MJ, Graffagnino C, Mcclenny C, *et al*. ApoE genotype and survival from intracerebral haemorrhage. Lancet, 1995,346(8974):575
34 Raffeld MR, Biffi A, Battey TW, *et al*. APOE epsilon4 and lipid levels affect risk of recurrent nonlobar intracerebral hemorrhage. Neurology, 2015,85(4):349-356

(Received May 15, 2018; revised Nov. 22, 2018)