Research Article

Apolipoprotein E ε4 Polymorphism as a Risk Factor for Ischemic Stroke: A Systematic Review and Meta-Analysis

Su-Ya Qiao, Ke Shang, Yun-Hui Chu, Hai-Han Yu, Xin Chen, Chuan Qin, Deng-Ji Pan, and Dai-Shi Tian

Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Correspondence should be addressed to Chuan Qin; qinchuan712@126.com, Deng-Ji Pan; djpan@tjh.tjmu.edu.cn, and Dai-Shi Tian; tiands@tjh.tjmu.edu.cn

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Introduction. Rising studies indicate that the apolipoprotein E (APOE) gene is related to the susceptibility of ischemic stroke (IS). However, certain consensus is limited by the lack of a large sample size of researches. This meta-analysis was performed to explore the potential association between the APOE gene and IS. Methods. To identify relevant case control studies in English publications by October 2020, we searched PubMed, Embase, Web of Science, and the Cochrane Library. Pooled odds ratios (ORs) with fixed- or random-effect models and corresponding 95% confidence intervals (CIs) were calculated to analyze potential associations. Results. A total of 55 researches from 32 countries containing 12207 IS cases and 27742 controls were included. The association between APOE gene ε4 mutation and IS was confirmed (ε4 vs. ε3 allele: pooled OR = 1.384, 95% CI, 1.281-2.060; ε2/ε4 vs. ε3/ε3: pooled OR = 1.233, 95% CI, 1.056-1.440; ε3/ε4 vs. ε3/ε3: pooled OR = 1.340, 95% CI, 1.165-1.542; ε4/ε4 vs. ε3/ε3: pooled OR = 1.833, 95% CI, 1.542-2.179; and APOE ε4 carriers vs. non-ε4 carriers: pooled OR = 1.377; 95% CI, 1.203-1.576). Interestingly, APOE ε4 mutation showed a dose-response correlation with IS risk (ε4/ε4 vs. ε2/ε4: pooled OR = 1.625; 95% CI, 1.281-2.060; ε4/ε4 vs. ε3/ε4: pooled OR = 1.301; 95% CI, 1.077-1.571). Similar conclusions were drawn in the small artery disease (SAD) subtype, but not in large artery atherosclerosis (LAA) or in cardioaortic embolism (CE), by subgroup analysis. Conclusions. These observations reveal that specific APOE ε4 mutation was significantly associated with the risk of IS in a dose-dependent manner, while APOE ε4 mutation was related to SAD subtype onset without a cumulative effect.

1. Introduction

Ischemic stroke (IS) is a disturbing problem worldwide, which is attributable to its leading role in disability and mortality worldwide, regardless of age, ethnicity, or gender [1]. Uncovering the etiology of IS is crucial for recognition and prevention of this disorder. Genetic elements and environmental components positively contribute to this multifactorial disease [2, 3]. Genetic inheritance provides a guide to the identification of high-risk individual. It deserves to investigate candidate gene polymorphisms in IS pathophysiological pathways. The apolipoprotein E (APOE) gene locates on chromosome 19q13.2. Two single polymorphisms (rs7412 and rs729358), three common alleles (ε2, ε3, and ε4), and six genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4) generate in populations [4]. The product of the APOE gene is a polymorphic protein named apolipoprotein E, which modulates the translocation of the cholesterol and other lipids among highly diverse cells [5], involved with neuroinflammation [6] and myelin integrity maintenance [7]. A study indicated that the activated CypA–MMP9 pathway in APOE4 carriers facilitated pericyte injury, which caused blood vessel dysfunction [8]. APOE polymorphisms and its risk associations with coronary artery disease [9], hypertension [10], diabetes [11], and carotid arterial atherosclerosis [12] are widely debated. The abovementioned diseases place individuals at a potential serious risk of IS. Individual studies of the association between IS and APOE polymorphisms have been explored extensively. Clinical differences, ethnic diversities, and small sample sizes restricted the present
finding to an inconsistent and controversial one. Previous meta-analyses concerning to this issue have been published several years ago [13] or limited to specific ethnicity [14, 15]. Accordingly, researches from 32 countries are qualified to form our meta-analysis to clarify how APOE genotypes are associated with IS. Moreover, we firstly revealed the correlation of the APOE gene and three IS subtypes (large artery atherosclerosis (LAA), small artery disease (SAD), and cardioaortic embolism (CE)).

2. Materials and Methods

We followed the rules of the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement to make this meta-analysis [16].

2.1. Data Availability. The data that contribute to the findings in our study are available and the corresponding authors can be contacted for data access.

2.2. Literature Search. Online databases (PubMed, Embase, Web of Science, and the Cochrane Library) were comprehensively searched for studies potentially involved and published in English publications and prior to October 30, 2020. We used a combination of some search terms relevant for IS (stroke, cerebral infarct, brain infarct, ischemic stroke, cerebral ischemia, transient ischemic attack, and cerebrovascular accident) and for the APOE gene (apolipoprotein E, APOE polymorphisms, apolipoprotein E polymorphisms, apolipoprotein E gene, rs429358, rs7412, apolipoprotein E epsilon 4, APOE e4, apolipoprotein E epsilon 2, and APOE e2). The detailed search strategies were showed next.

2.3. Selection Criteria. The selection of the studies was independently completed by two investigators, and any difference was resolved by discussion until an agreement was reached. We carefully selected case control studies that evaluated the relationship of the APOE gene and IS with definite IS diagnoses (using computed tomography, magnetic resonance, or autopsy) regardless of the ethnic background. The detailed inclusion criteria were (1) high-quality studies which explore the relationship between the APOE polymorphisms and IS; (2) explicit IS diagnostic criteria, (3) nonstroke individuals as the control group, and (4) original data including independent and sufficient APOE genotype data, to compute ORs and 95% CI. The newest and largest studies were chosen to avoid duplicate or overlapped data information.

2.4. Data Extraction. Two investigators separately finished full-text reading to extract the needed information from each selected study and resolved the controversial items through serious discussion. The extracted information was (1) research characteristics, including the first author’s name, year of publication, and geographical location of the study; (2) participant details, such as the sex ratio, mean age, and the sample size of case and control groups; (3) diagnostic criteria for IS; (4) determination methods of the APOE gene; (5) each genotype frequency; (6) the sample sizes of IS subtypes according to TOAST norms and respective genotype frequency; and (7) HWE in controls.

2.5. Quality Assessment. We performed the quality assessment through the Newcastle-Ottawa Scale (NOS) score considering selection, comparability, and exposure. It ranged from 0 (worst) to 9 (best) and high-quality studies were known as with a NOS score ≥ 7.

2.6. Statistical Analysis. We performed Stata 14.0 to complete all data analyses. The chi-square test was used to examine the Hardy-Weinberg equilibrium (HWE) in control groups. An overt deviation from HWE was regarded as P < 0.05. The compositive ORs and 95% CI were calculated. We explored five genetic models to generate the respective pooled ORs: (1) allele comparisons (e2 allele vs. e3 allele; e4 allele vs. e3 allele); (2) genotype comparisons (e2/e2 vs. e3/e3; e2/e3 vs. e3/e3; e2/e4 vs. e3/e3; e3/e4 vs. e3/e3; e4/e4 vs. e3/e3); (3) APOE e4 carrier comparisons: we defined three e4-containing genotypes (e2/e4 + e3/e4 + e4/e4) as APOE e4 carriers and the other genotypes (e2/e2 + e2/e3 + e3/e3) as non-APOE e4 carriers; (4) APOE e2 carrier comparisons: similar comparisons of e2-containing genotypes (e2/e2 + e2/e3 + e2/e4) vs. non-e2-containing genotypes (e3/e3 + e3/e4 + e4/e4); and (5) comparisons between APOE e4 homozygosis and e4 heterozygote (e4/e4 vs. e2/e4; e4/e4 vs. e3/e4). The I² statistic and Cochran’s Q test were applied to measure the heterogeneity between studies [17]. We selected the random effect model (DerSimonian-Laird method) when no heterogeneity was found between studies (I² < 50.0%) and fixed-effect model (Mantel-Haenszel method) when no heterogeneity existed (I² ≥ 50.0%). Subgroup analysis was conducted to confirm the relationship between the APOE polymorphisms and the risk of different IS subgroups. Sensitivity analysis was performed by successively removing a single study one by one to verify the stability and reliability of our conclusions. Meta-regression analysis was operated to recognize sources of heterogeneity. Funnel plots and quantified Egger’s tests were accomplished to test publication bias. Significant publication bias was considered as the P value of Egger’s test less than 0.10 or obvious asymmetric funnel plot.

2.7. The Result of Trial Sequential Analysis (TSA). Insufficient sample size, continuous updating, and repeating “significance testing” could increase the risk of type I errors. Therefore, traditional meta-analysis that focuses on the specific topic may suffer an increased risk of random error. Trial sequential analysis (TSA) was used to reduce the risk of type I error and obtain important information regarding the required sample size for such trials. Set the time sequence of a single study as the research node, and then, perform an interim analysis between the new study that will be included in meta-analysis and existing data accumulation. The required information size (RIS), trial sequential monitoring boundary, and futility boundary are estimated using the TSA. As the sample size of meta-analysis reaching the RIS or the z-curve crossing the trial sequential monitoring boundary, we can conclude that the results of meta-analysis are quite stable and further studies were not needed. We accomplished TSA following the guidelines of the user manual and previous article [18] by setting a
significance of 5% for type I error, a relative risk reduction of 20%, and a statistical test power of 80% with TSA software (TSA, version 0.9 beta; Copenhagen Trial Unit, Copenhagen, Denmark).

3. Results

3.1. Characteristics of Eligible Studies. We collect a total of 55 studies from 32 countries containing 12207 IS cases and 27742 controls to make the meta-analysis [19–73]. Figure 1 showed the detailed selection process. The selected studies and their main characteristics were exhibited in Table 1. Fifteen of the studies provided data about different subtypes (grouped by classification of cerebrovascular diseases III or TOAST classification) of IS: large artery atherosclerosis (LAA), small artery disease (SAD), and cardioaortic embolism (CE). We extracted them independently and specific information was showed in supplementary material table 1. There were seven studies (Koopal et al. 2016, Lai et al. 2007, Chowdhury et al. 2001, Kokubo et al. 2000, Ji et al. 1998, Couderc et al. 1993, Saidi et al. 2009) which deviated HWE obviously, and one study (Schneider et al. 2005) did not contain enough data to obtain HWE. Forty-eight studies used PCR-based method and seven researches (Slowik et al. 2003, Karttunen et al. 2002, Hachinski et al. 1996, Couderc et al. 1993, Brewin et al. 2020, Aalto-Setala et al. 1998, Schneider et al. 2005) used other methods to identify APOE genotypes. These studies used computed tomography or magnetic resonance to diagnose IS except that one research which used autopsy (Schneider et al. 2005). The NOS score mean value was 7.509, which suggested that the quality of included studies was reliable (supplementary material Table 2). PRISMA2020 checklist was provided to present our meta-analysis items (supplementary material Table 3).

3.2. Main Results of the Comparisons in the Abovementioned Five Genetic Models

3.2.1. Allele Comparisons. In comparison with the ε3 allele, the ε2 allele did not show association of the risk of IS (pooled OR = 0.983, 95% CI, 0.867-1.115, P = 0.79) (as showed in Table 2), while the ε4 allele contributed to an obviously increased risk of IS with the pooled OR = 1.374 (95% CI, 1.214-1.556, P < 0.0001) (Figure 2(d)).

3.2.2. Genotype Comparisons. When compared with the ε3/ε3 genotype, the pooled effects of the APOE genotype in the meta-analysis were as follows: for the ε2/ε2 genotype, pooled OR = 0.985, 95% CI, 0.653-1.486, P = 0.94, and for the ε2/ε3 genotype, pooled OR = 0.980, 95% CI, 0.900-1.066, P = 0.63; those two genotypes presented no association with the risk of IS (as showed in Table 2). Genotypes ε2/ε4, ε3/ε4, and ε4/ε4 were related to a higher risk of IS than ε3/ε3. The respective IS risk ORs were 1.233 (95% CI, 1.056-1.440, P = 0.01) (Figure 2(a)), 1.340 (95% CI, 1.165-1.542, P < 0.0001) (Figure 2(b)), and 1.833 (95% CI, 1.542-2.179, P < 0.0001) (Figure 2(c)). The above results could be found in Table 2. A conclusion was drawn: every genotype which contained APOE ε4 mutation increased the risk of IS.

3.2.3. APOE ε4 Carrier Comparisons. Compared with the non-ε4 carriers, we confirmed that the ε4 carriers were associated with the increased risk of IS; the pooled outcome was pooled OR = 1.377 (95% CI, 1.203-1.576, P < 0.0001) (Figure 2(e)).
| Study ID          | Region     | Criteria for IS | Genotyping method | Source of control | Group | Sample size | Male/n (%) | Characteristics and the counts of every genotype |   |   |   |   |   |   |   |   |   |   |   |   |
|------------------|------------|-----------------|-------------------|-------------------|-------|-------------|------------|-----------------------------------------------|---|---|---|---|---|---|---|---|---|---|---|
| Wu et al., 2020  | China      | CT/MRI          | PCR               | H-B               | Case  | 938         | 581 (61.9%) | ε2/ε2: 2/6, ε2/ε3: 63/18, ε2/ε4: 684/156, ε3/ε4: 15/5 | 85 | 1587 | 204 | Y | 8 |
|                  |            |                 |                   |                   | Control | 1028       | 622 (60.5%) | 63.7 ± 12.4, ε2/ε2: 9/13, ε2/ε3: 13/7, ε2/ε4: 763/106, ε3/ε3: 106/6 | 162 | 1763 | 131 |
| Zhao et al., 2017| China      | CT/MRI          | PCR               | H-B               | Case  | 513         | 294 (57.3%) | 62.3 ± 12.2, ε2/ε2: 3/6, ε2/ε3: 7/3, ε2/ε4: 347/85, ε3/ε3: 8/7 | 76 | 842 | 108 | Y | 7 |
|                  |            |                 |                   |                   | Control | 514       | 288 (56.0%) | 61.7 ± 13.5, ε2/ε2: 5/7, ε2/ε3: 7/8, ε2/ε4: 366/64, ε3/ε3: 1/1 | 88 | 866 | 74 |
| Coen Herak et al., 2017 | Croatia | CT/MRI | PCR | P-B | Case  | 73    | 48 (65.8%)  | 4.3 ± X 0/10 2/50 11/0 12/121 13/13 | Y | 8 |
|                  |            |                 |                   |                   | Control | 100   | 63 (65.0%)  | 6.5 ± X 1/11 7/74 13/13 1/13 172/15 |
| Das et al., 2016 | India      | CT/MRI          | PCR-RFLP          | P-B               | Case  | 620         | 434 (70.0%) | 49.4 ± 17.4, ε2/ε2: 5/46 6/431 ε2/ε3: 120/12 ε3/ε3: 62/1028 | 150 | Y | 8 |
|                  |            |                 |                   |                   | Control | 620   | 428 (69.0%) | 49.1 ± 16.9, ε2/ε2: 5/50 4/436 ε2/ε3: 113/12 ε3/ε3: 64/1035 | 141 |
| Koopal et al., 2016 | Netherlands | CT | PCR | P-B | Case  | 278   | NA | NA 3/30 8/160 9/8 44 419/93 |
|                  |            |                 |                   |                   | Control | 4220 | NA | NA 50/389 96/2422 1127/136 585/636 1495 |
| Luo et al., 2015 | China      | CT/MRI          | PCR               | H-B               | Case  | 712         | 465 (65.3%) | 65.2 ± 13.9, ε2/ε2: 4/93 13/494 ε2/ε3: 101/7 114/1182 | 128 | Y | 7 |
|                  |            |                 |                   |                   | Control | 774   | 418 (54.0%) | 51.5 ± 16.9, ε2/ε2: 3/107 8/535 ε2/ε3: 113/8 121/1290 | 137 |
| Wei et al., 2015 | Malaysia   | CT/MRI          | PCR               | P-B               | Case  | 297         | 33 (11.1%)  | 52.6 ± 8.8, ε2/ε2: 8/68 23/137 54/7 107/396 | 91 | Y | 8 |
|                  |            |                 |                   |                   | Control | 297   | 119 (40.0%) | 51.8 ± 8.7, ε2/ε2: 4/12 27/163 89/2 47/427 | 120 |
| Yan et al., 2015 | China      | CT/MRI          | PCR-RFLP          | H-B               | Case  | 580         | 387 (66.7%) | 59.8 ± 13.7, ε2/ε2: 11/41 33/351 82/62 96/825 | 239 | Y | 8 |
|                  |            |                 |                   |                   | Control | 580   | 379 (65.3%) | 59.4 ± 13.1, ε2/ε2: 61/54 49/354 33/29 225/795 | 140 |
| Chatzistefanidis et al., 2014 | Greece | CT/MRI | PCR | H-B | Case  | 329    | 225 (68.4%) | 59.7 ± 11.6, ε2/ε2: 3/36 3/227 56/4 45/546 | 67 | Y | 7 |
|                  |            |                 |                   |                   | Control | 361   | 205 (56.8%) | 60.4 ± 13.7, ε2/ε2: 2/24 8/278 47/2 36/627 | 59 |
| Study ID                        | Region   | Criteria for IS | Genotyping method | Source of control | Case | Control | Male/\(n\) (\%) | Age(years) | \(\epsilon_2/\epsilon_2\) | \(\epsilon_2/\epsilon_3\) | \(\epsilon_2/\epsilon_4\) | \(\epsilon_3/\epsilon_3\) | \(\epsilon_3/\epsilon_4\) | \(\epsilon_4/\epsilon_4\) | \(H\) | \(N\) |
|-------------------------------|----------|-----------------|-------------------|-------------------|------|---------|-----------------|------------|-----------------|-----------------|----------------|----------------|----------------|----------------|------|
| Atadzhanov et al., 2013 [28]  | Zambia   | CT/PCR          | P-B               | Case             | 23   | NA      | 54.0 ± 16.0     | 0          | 3               | 3               | 7               | 0               | 7               | 29              | 10   | Y    |
|                              |          |                 |                   | Control          | 116  | NA      | 50 (41.4%)      | 0          | 25              | 7               | 38              | 37              | 9               | 32              | 138 | 62  |
| Gelfand et al., 2013 [29]    | America  | CT/MRI PCR-RFLP | H-B               | Case             | 13   | NA      | 10 (77.0%)      | 0          | 1               | 2               | 5               | 3               | 2               | 3               | 14  | 9    |
|                              |          |                 |                   | Control          | 84   | NA      | 46 (55.0%)      | 0          | 8               | 3               | 55              | 16              | 2               | 11              | 134 | 23  |
| Balcerzyk et al., 2010 [30]  | Poland   | CT/MRI PCR      | P-B               | Case             | 72   | NA      | 8.8 ± 5.6       | 1          | 9               | 0               | 52              | 6               | 4               | 11              | 119 | 14  |
|                              |          |                 |                   | Control          | 71   | NA      | 8.2 ± 5.4       | 0          | 8               | 0               | 51              | 11              | 1               | 8               | 121 | 13  |
| Tamam et al., 2009 [31]      | Turkey   | CT/MRI PCR      | H-B               | Case             | 65   | NA      | 61.9 ± 14.7     | 0          | 1               | 1               | 25              | 2               | 1               | 2               | 53  | 5    |
|                              |          |                 |                   | Control          | 30   | NA      | 61.9 ± 14.7     | 0          | 2               | 1               | 25              | 2               | 1               | 2               | 53  | 5    |
| Tascilar et al., 2009 [32]   | Turkey   | CT/MRI PCR      | P-B               | Case             | 85   | NA      | 61.7 ± 13.6     | 3          | 18              | 3               | 45              | 9               | 7               | 27              | 117 | 26  |
|                              |          |                 |                   | Control          | 77   | NA      | 54.7 ± 8.4      | 3          | 16              | 7               | 40              | 9               | 2               | 29              | 105 | 20  |
| Wang et al., 2009 [33]       | China    | CT/MRI PCR      | H-B               | Case             | 396  | NA      | 57.3 ± 8.2      | 16         | 98              | 60              | 124             | 87              | 11              | 190             | 433 | 169 |
|                              |          |                 |                   | Control          | 396  | NA      | 57.3 ± 8.3      | 33         | 116             | 41              | 164             | 39              | 3               | 223             | 483 | 86  |
| Lai et al., 2007 [34]        | China    | MRI PCR         | H-B               | Case             | 257  | NA      | 63.7 ± 8.2      | 1          | 17              | 10              | 162             | 67              | 0               | 29              | 408 | 77  |
|                              |          |                 |                   | Control          | 112  | NA      | 71.0 ± 10.6     | 4          | 5               | 5               | 78              | 19              | 1               | 18              | 180 | 26  |
| Parfenov et al., 2007 [35]   | Yakutsk  | CT/MRI PCR      | P-B               | Case             | 107  | NA      | 58.4 ± 11.5     | 1          | 5               | 1               | 63              | 33              | 4               | 8               | 164 | 42  |
|                              |          |                 |                   | Control          | 101  | NA      | 57.6 ± 11.6     | 1          | 15              | 3               | 58              | 22              | 2               | 20              | 153 | 29  |
| Kang and Lee, 2006 [36]      | Korea    | MRI PCR         | H-B               | Case             | 194  | NA      | 62.0 ± 9.5      | 0          | 24              | 0               | 126             | 44              | 0               | 24              | 320 | 44  |
|                              |          |                 |                   | Control          | 168  | NA      | 62.3 ± 6.3      | 2          | 18              | 0               | 128             | 19              | 1               | 22              | 293 | 21  |
| Study ID | Region | Criteria for IS | Genotyping method | Source of control | Sample size | Male/n (%) | Age (years) | ε2/ε2 | ε2/ε3 | ε2/ε4 | ε3/ε3 | ε3/ε4 | ε4/ε4 | H  | N |
|----------|--------|----------------|-------------------|-------------------|-------------|------------|-------------|--------|--------|--------|--------|--------|--------|----|---|
| Gao et al., 2006 [37] | China | CT/MRI | PCR | H-B | Case 100 | 71 (71.0%) | 61.1 ± 10.8 | 1 | 11 | 0 | 75 | 13 | 0 | 13 | 174 | 13 | Y 8 |
| | | | | | Control 100 | 71 (71.0%) | 61.0 ± 10.6 | 1 | 13 | 0 | 80 | 6 | 0 | 15 | 179 | 6 | |
| Baum et al., 2006 [38] | China | CT/MRI | PCR | P-B | Case 243 | 134 (54.5%) | 70.7 ± 12.0 | 7 | 39 | 6 | 155 | 32 | 4 | 59 | 381 | 46 | Y 8 |
| | | | | | Control 311 | 152 (45.2%) | 70.0 ± 5.9 | 2 | 60 | 6 | 203 | 39 | 1 | 70 | 505 | 47 | |
| Pezzini et al., 2005 [39] | Italy | CT/MRI | PCR | H-B | Case 163 | 84 (51.5%) | 35.0 ± 7.5 | 2 | 12 | 1 | 109 | 38 | 1 | 17 | 268 | 41 | Y 8 |
| | | | | | Control 158 | 85 (53.8%) | 34.8 ± 6.1 | 0 | 16 | 1 | 120 | 21 | 0 | 17 | 277 | 22 | |
| Cerrato et al., 2005 [40] | Italy | CT/MRI | PCR | P-B | Case 302 | 100 (33.1%) | 57.0 ± 11.0 | 9 | 31 | 0 | 230 | 28 | 4 | 49 | 519 | 36 | Y 7 |
| | | | | | Control 228 | 104 (33.1%) | 55.0 ± 16.0 | 3 | 25 | 1 | 158 | 37 | 4 | 32 | 387 |
| Jin et al., 2004 [41] | China | CT/MRI | PCR-RFLP | P-B | Case 226 | 129 (57.1%) | 48.5 ± 3.4 | 2 | 14 | 3 | 152 | 52 | 3 | 21 | 370 | 61 | Y 8 |
| | | | | | Control 201 | 109 (54.2%) | 47.1 ± 2.4 | 2 | 17 | 2 | 156 | 22 | 2 | 23 | 351 | 28 | |
| Duzenli et al., 2004 [42] | Turkey | CT | PCR | P-B | Case 62 | NA | NA | 0 | 8 | 1 | 52 | 1 | 0 | 9 | 113 | 2 | Y 8 |
| | | | | | Control 126 | 61 (48.4%) | 58.0 ± 1.9 | 2 | 23 | 2 | 80 | 18 | 1 | 29 | 201 | 22 | |
| Slowik et al., 2003 [43] | Poland | CT/MRI | Immuno-blotting | H-B | Case 71 | 49 (69.0%) | 59.6 ± 9.5 | 0 | 3 | 0 | 53 | 14 | 1 | 3 | 123 | 16 | Y 7 |
| | | | | | Control 30 | 39 (63.4%) | 63.1 ± 8.8 | 0 | 1 | 0 | 21 | 8 | 0 | 1 | 51 | 8 | |
| Souza et al., 2003 [44] | Brazil | CT | PCR | P-B | Case 107 | NA | NA | 68.8 ± 9.2 | 0 | 5 | 0 | 93 | 8 | 1 | 5 | 199 | 10 | Y 8 |
| | | | | | Control 100 | NA | NA | 69.4 ± 8.3 | 0 | 8 | 2 | 74 | 16 | 0 | 10 | 172 | 18 | |
| Karttunen et al., 2002 [45] | Finland | CT/MRI | Immuno-blotting | P-B | Case 44 | 27 (61.4%) | 15–60 | 0 | 3 | 1 | 27 | 13 | 0 | 4 | 70 | 14 | Y 8 |
| | | | | | Control 104 | 59 (56.7%) | 15–60 | 1 | 4 | 1 | 67 | 28 | 3 | 7 | 166 | 35 | |
| Morrison et al., 2002 [46] | America | MRI | PCR | P-B | Case 400 | NA | NA | 1 | 48 | 19 | 199 | 118 | 15 | 69 | 564 | 167 | Y 7 |
| Study ID | Region       | Criteria for IS | Genotyping method | Source of control | Group | Sample size | Male (%) | Age (years) | e2 | e2 | e3 | e3 | e4 | e4 | e2 | e2 | e3 | e3 | e4 | e4 | H | N |
|---------|--------------|-----------------|-------------------|-------------------|-------|-------------|----------|-------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|---|
| MacLeod et al, 2001 [47] | Scotland | CT | PCR | P-B | Case | 266 | 150 (56.4%) | 65.7 ± 12.2 | 1 | 29 | 7 | 170 | 56 | 3 | 38 | 425 | 69 | Y | 7 |
| Chowdhury et al, 2001 [48] | Bangladesh | CT | PCR | H-B | Case | 147 | 116 (79.9%) | 57.9 ± 11.1 | 3 | 3 | 0 | 113 | 26 | 2 | 9 | 255 | 30 | N | 6 |
| Frikke-Schmidt et al, 2001 [49] | Denmark | CT | PCR | P-B | Case | 738 | 282 (61.8%) | 63.0 ± 7.4 | 5 | 77 | 23 | 409 | 207 | 17 | 110 | 1102 | 264 | Y | 6 |
| Catto et al, 2000 [50] | England | CT | PCR | P-B | Case | 515 | 259 (50.3%) | 73.0 ± X | 0 | 61 | 8 | 321 | 115 | 10 | 69 | 818 | 143 | Y | 7 |
| Kokubo et al, 2000 [51] | Japan | CT/MRI | PCR-RFLP | P-B | Case | 201 | NA | 40–89 | 12 | 15 | 2 | 138 | 33 | 1 | 41 | 324 | 37 | N | 7 |
| Peng et al, 1999 [52] | China | CT | PCR | H-B | Case | 90 | NA | 62.6 ± 8.9 | 0 | 13 | 1 | 55 | 19 | 2 | 14 | 142 | 24 | Y | 7 |
| Ji et al, 1998 [53] | Japan | CT/MRI | PCR-RFLP | P-B | Case | 123 | NA | 70.2 ± 7.2 | 0 | 9 | 3 | 79 | 29 | 3 | 12 | 196 | 38 | N | 7 |
| Margaglione et al, 1998 [54] | Italy | CT/MRI | PCR | P-B | Case | 100 | 51 (51.0%) | 66.2 ± 10.0 | 1 | 10 | 0 | 59 | 24 | 6 | 12 | 152 | 36 | Y | 8 |
| Kessler et al, 1997 [55] | Germany | CT/MRI | PCR | H-B | Case | 227 | 108 (47.6%) | 62.3 ± 14.2 | 2 | 31 | 5 | 132 | 50 | 7 | 40 | 345 | 69 | Y | 8 |
| Hachinski et al, 1996 [56] | Britain | CT/MRI | IF | P-B | Case | 89 | 61 (67.8%) | 64.6 ± 8.7 | 1 | 13 | 1 | 47 | 24 | 3 | 16 | 131 | 31 | Y | 8 |
| Study ID           | Region  | Criteria for IS | Genotyping method | Source of control | Group | Sample size | Characteristics and the counts of every genotype | H | N |
|-------------------|---------|-----------------|--------------------|-------------------|-------|-------------|-----------------------------------------------|---|---|
| Couderc et al., 1993 [57] | France | CT              | IF                 | H-B               | Case 69 | 36 (52.2%) | 72.3 ± 11.6 1 7 0 50 10 1 9 1 117 12 N 7 |   |   |
|                   |         |                 |                    |                   | Control 566 | 347 (61.3%) | 41.3 ± 15.3 8 60 5 377 109 7 81 923 128 |   |   |
| Qian et al., 2012 [58] | China  | CT/MRI          | PCR                | H-B               | Case 152 | 87 (57.2%) | 66.8 ± 5.5 0 21 0 95 29 7 21 240 43 Y 9 |   |   |
|                   |         |                 |                    |                   | Control 40 | 13 (32.5%) | 64.0 ± 12.6 0 5 0 29 6 0 5 69 6 |   |   |
| Konialis et al., 2016 [59] | Greece | CT              | PCR                | H-B               | Case 200 | 142 (72.0%) | 60.0 ± 16.0 0 10 3 145 39 3 13 339 48 Y 7 |   |   |
|                   |         |                 |                    |                   | Control 159 | 76 (47.5%) | 59.0 ± 13.0 1 16 0 126 16 0 18 284 16 |   |   |
| Fayad et al., 2009 [60] | Egypt  | CT/MRI          | PCR-RFLP           | H-B               | Case 40 | NA          | NA 0 3 7 11 11 8 10 36 34 Y 6 |   |   |
|                   |         |                 |                    |                   | Control 20 | NA          | NA 0 3 1 15 1 0 4 34 2 |   |   |
| Stankovic et al., 2004 [61] | Serbian | CT/MRI         | PCR-RFLP           | P-B               | Case 65 | NA          | NA 0 6 0 39 18 2 6 102 22 Y 7 |   |   |
| Pedro-Botet et al., 1992 [62] | Spain  | CT              | PCR                | P-B               | Case 100 | NA          | NA 0 12 0 54 26 6 16 146 38 Y 7 |   |   |
|                   |         |                 |                    |                   | Control 100 | NA          | NA 0 13 2 69 13 3 15 164 21 |   |   |
| Fekih-Mrissa et al., 2014 [63] | Tunisia | CT/MRI          | PCR                | P-B               | Case 6 | NA          | NA 0 0 0 0 5 1 0 5 7 |   |   |
| Brewin et al., 2020 [64] | London | CT/MRI          | Exome sequencing    | P-B               | Case 47 | NA          | NA 0 5 8 14 14 6 13 47 34 Y 7 |   |   |
|                   |         |                 |                    |                   | Control 236 | NA          | NA 6 41 11 97 71 10 64 306 102 |   |   |
| Saidi et al., 2009 [65] | Tunisia | CT/MRI          | PCR                | P-B               | Case 228 | 114 (50.0%) | 61.5 ± 12.1 0 14 25 74 87 28 39 249 168 Y 8 |   |   |
|                   |         |                 |                    |                   | Control 323 | 177 (54.8%) | 60.9 ± 12.8 0 27 28 187 71 10 55 472 119 |   |   |
| Wen et al., 2006 [66] | China   | MRI             | PCR                | P-B               | Case 67 | NA          | NA 7.0 ± 11.4 4 7 2 41 11 2 17 100 17 Y 9 |   |   |
|                   |         |                 |                    |                   | Control 134 | NA          | NA 2 24 3 89 15 1 31 217 20 |   |   |
| Giassakis et al., 2007 [67] | Greece | CT/MRI          | PCR                | P-B               | Case 100 | 70 (70.0%) | 60.7 ± 9.8 NA NA NA NA NA NA NA 12 166 22 Y 8 |   |   |
|                   |         |                 |                    |                   | Control 96 | 66 (68.8%) | 61.3 ± 9.8 NA NA NA NA NA NA NA 10 169 13 |   |   |
| Study ID                  | Region | Criteria for IS | Genotyping method | Source of control | Group  | Sample size | Characteristics and the counts of every genotype | H  | N  |
|---------------------------|--------|-----------------|-------------------|-------------------|--------|-------------|------------------------------------------------|-----|----|
| Nakata et al., 1997 [68]  | Japan  | CT/MRI          | PCR               | P-B               | Case   | 55          | 25 (45.0%) Age (years) 66.0 ± 14.0 NA NA NA NA NA 2 98 10 Y 7 |     |    |
|                           |        |                 |                   |                   | Control| 61          | 30 (49.0%) Age (years) 67.0 ± 8.0 NA NA NA NA NA 7 110 5 |     |    |
| Szolnoki et al., 2002 [69]| Hungary| MRI             | PCR               | H-B               | Case   | 689         | 356 (51.7%) Age (years) 59.8 ± 17.7 NA NA NA NA NA 104 934 340 Y 7 |     |    |
|                           |        |                 |                   |                   | Control| 652         | 341 (52.3%) Age (years) 59.8 ± 16.9 NA NA NA NA NA 118 1016 170 |     |    |
| Aalto-Setala et al., 1998 [70] | Finland | CT/MRI          | IF                | P-B               | Case   | 231         | <60 Age (years) NA NA NA NA NA 17 350 95 Y 7 |     |    |
|                           |        |                 |                   |                   | Control| 615         | NA Age (years) 20–55 NA NA NA NA NA 74 861 295 |     |    |
| Artieda et al., 2008 [71] | Spain  | CT/MRI          | PCR               | P-B               | Case   | 152         | NA Age (years) 61.7 ± 6.8 $\epsilon_2/\epsilon_2 + \epsilon_2/\epsilon_3 = 15$ 1 110 $\epsilon_3/\epsilon_4 + \epsilon_4/\epsilon_4 = 26$ NA NA NA Y 7 |     |    |
|                           |        |                 |                   |                   | Control| 215         | NA Age (years) NA $\epsilon_2/\epsilon_2 + \epsilon_2/\epsilon_3 = 20$ 1 164 $\epsilon_3/\epsilon_4 + \epsilon_4/\epsilon_4 = 30$ NA NA NA |     |    |
| Schneider et al., 2005 [72]| America| CT              | PCR               | P-B               | Case   | 76          | NA Age (years) $\epsilon_2/\epsilon_2 = 0; \epsilon_2/\epsilon_3 + \epsilon_3/\epsilon_3 = 45; \epsilon_2/\epsilon_4 + \epsilon_3/\epsilon_4 + \epsilon_4/\epsilon_4 = 31$ NA NA NA 7 |     |    |
|                           |        |                 |                   |                   | Control| 138         | NA Age (years) NA $\epsilon_2/\epsilon_2 = 0; \epsilon_2/\epsilon_3 + \epsilon_3/\epsilon_3 = 104; \epsilon_2/\epsilon_4 + \epsilon_3/\epsilon_4 + \epsilon_4/\epsilon_4 = 34$ NA NA NA |     |    |
| Li et al., 2016 [73]      | China  | CT/MRI          | PCR               | P-B               | Case   | 164         | 113 (68.9%) Age (years) 60.8 ± 11.9 $\epsilon_2/\epsilon_2 + \epsilon_2/\epsilon_3 + \epsilon_3/\epsilon_3 = 110; \epsilon_2/\epsilon_4 + \epsilon_3/\epsilon_4 + \epsilon_4/\epsilon_4 = 42; \epsilon_4/\epsilon_4 = 12$ NA NA NA Y 8 |     |    |
|                           |        |                 |                   |                   | Control| 109         | 64 (58.7%) Age (years) 59.4 ± 13.0 $\epsilon_2/\epsilon_2 + \epsilon_2/\epsilon_3 + \epsilon_3/\epsilon_3 = 85; \epsilon_2/\epsilon_4 + \epsilon_3/\epsilon_4 + \epsilon_4/\epsilon_4 = 22; \epsilon_4/\epsilon_4 = 2$ NA NA NA |     |    |

*Age (years): different statistical patterns of age (mean and IQR, mean ± SD, or range) were extracted. CT: computerized tomography; MRI: magnetic resonance imaging; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism; IS: ischemic stroke; H: Hardy-Weinberg equilibrium; N: Newcastle-Ottawa Scale; NA: not available; IF: isoelectric focusing; H-B: hospital based; P-B: population based.
| Genetic model of APOE gene polymorphisms | Group | No. of included studies | OR     | 95% CI             | P value of ORs |
|----------------------------------------|-------|------------------------|--------|--------------------|----------------|
| ε2 allele vs. ε3 allele                | All   | 51                     | 0.983  | (0.867,1.115)      | 0.79           |
|                                        | LAA   | 13                     | 0.962  | (0.712,1.299)      | 0.80           |
|                                        | CE    | 10                     | 1.517  | (0.861,2.674)      | 0.15           |
|                                        | SAD   | 12                     | 1.190  | (0.997,1.421)      | 0.05           |
| ε4 allele vs. ε3 allele                | All   | 51                     | 1.374  | (1.214,1.556)      | <0.0001        |
|                                        | LAA   | 13                     | 1.149  | (0.898,1.469)      | 0.27           |
|                                        | CE    | 10                     | 1.092  | (0.662,1.801)      | 0.73           |
|                                        | SAD   | 12                     | 1.318  | (1.073,1.618)      | 0.01           |
| ε2/ε2 vs. ε3/3                        | All   | 36                     | 0.985  | (0.653,1.486)      | 0.94           |
|                                        | LAA   | 11                     | 1.307  | (0.750,2.278)      | 0.19           |
|                                        | CE    | 10                     | 4.290  | (1.917,9.600)      | <0.0001        |
|                                        | SAD   | 11                     | 1.803  | (1.037,3.134)      | 0.04           |
| ε2/ε3 vs. ε3/3                        | All   | 46                     | 1.233  | (1.056,1.440)      | 0.01           |
|                                        | LAA   | 13                     | 0.869  | (0.705,1.071)      | 0.19           |
|                                        | CE    | 10                     | 1.255  | (0.849,1.856)      | 0.26           |
|                                        | SAD   | 12                     | 1.178  | (0.952,1.457)      | 0.13           |
| ε2/ε4 vs. ε3/3                        | All   | 42                     | 1.340  | (1.165,1.542)      | <0.0001        |
|                                        | LAA   | 11                     | 0.978  | (0.607,1.576)      | 0.93           |
|                                        | CE    | 10                     | 1.458  | (0.534,3.980)      | 0.46           |
|                                        | SAD   | 10                     | 0.932  | (0.526,1.652)      | 0.81           |
| ε3/ε4 vs. ε3/3                        | All   | 47                     | 1.833  | (1.542,2.179)      | <0.0001        |
|                                        | LAA   | 14                     | 1.154  | (0.841,1.584)      | 0.38           |
|                                        | CE    | 10                     | 1.175  | (0.627,2.203)      | 0.62           |
|                                        | SAD   | 13                     | 1.392  | (1.097,1.767)      | 0.01           |
| ε4/ε4 vs. ε3/3                        | All   | 46                     | 1.833  | (1.542,2.179)      | <0.0001        |
|                                        | LAA   | 13                     | 1.367  | (0.836,2.236)      | 0.21           |
|                                        | CE    | 10                     | 1.543  | (0.591,4.029)      | 0.38           |
|                                        | SAD   | 11                     | 1.809  | (1.030,3.175)      | 0.04           |
| ε4 vs. non-ε4                         | All   | 50                     | 1.377  | (1.203,1.576)      | <0.0001        |
|                                        | LAA   | 14                     | 1.149  | (0.876,1.506)      | 0.32           |
|                                        | CE    | 10                     | 1.091  | (0.645,1.845)      | 0.74           |
|                                        | SAD   | 13                     | 1.329  | (1.064,1.661)      | 0.01           |
| ε2 vs. non-ε2                         | All   | 48                     | 0.956  | (0.841,1.086)      | 0.49           |
|                                        | LAA   | 14                     | 0.861  | (0.717,1.035)      | 0.11           |
|                                        | CE    | 10                     | 1.358  | (0.966,1.910)      | 0.08           |
|                                        | SAD   | 13                     | 1.117  | (0.926,1.347)      | 0.25           |
| ε4/ε4 vs. ε2/4                        | All   | 40                     | 1.625  | (1.281,2.060)      | <0.0001        |
|                                        | LAA   | 11                     | 1.551  | (0.791,3.043)      | 0.20           |
|                                        | CE    | 9                      | 0.771  | (0.177,3.352)      | 0.73           |
|                                        | SAD   | 4                      | 2.115  | (0.919,4.867)      | 0.08           |
| ε4/ε4 vs. ε3/4                        | All   | 46                     | 1.301  | (1.077,1.571)      | 0.01           |
|                                        | LAA   | 13                     | 1.353  | (0.811,2.258)      | 0.25           |
|                                        | CE    | 6                      | 1.077  | (0.402,2.887)      | 0.88           |
|                                        | SAD   | 11                     | 1.332  | (0.739,2.400)      | 0.34           |
## Study ID OR (95% CI) % Weight

| Study ID               | OR (95% CI)         | %  | Weight |
|-----------------------|---------------------|----|--------|
| Wu et al. 2020        | 1.545 (0.751, 3.176) | 4.27 | 100.00 |
| Zhao et al. 2017      | 0.923 (0.331, 2.572) | 2.71 |        |
| Coen Herak et al. 2017| 7.376 (0.347, 156.894)| 0.14|        |
| Das et al. 2016       | 1.517 (0.425, 5.415) | 1.39 |        |
| Koopal et al. 2016    | 1.261 (0.603, 2.641) | 4.06 |        |
| Luo et al. 2015       | 1.760 (0.723, 4.282) | 2.67 |        |
| Wei et al. 2015       | 1.014 (0.556, 1.848) | 7.50 |        |
| Chatzistefanidis et al. 2014 | 0.459 (0.120, 1.751) | 2.50 |        |
| Atadzhanov et al. 2013| 1.810 (0.390, 8.401) | 0.78 |        |
| Gelfand et al. 2013   | 7.333 (0.983, 54.721)| 0.16|        |
| Tamam et al. 2009     | 1.000 (0.086, 11.565)| 0.45|        |
| Brewin et al. 2020    | 5.039 (1.730, 14.680)| 0.84|        |
| Yan et al. 2015       | 0.679 (0.426, 1.082) | 15.51|        |
| Saidi et al. 2009     | 2.256 (1.235, 4.123) | 4.68 |        |
| Tascliar et al. 2009  | 0.381 (0.092, 1.573) | 2.35 |        |
| Wang et al. 2009      | 1.935 (1.221, 3.068) | 9.27 |        |
| Lai et al. 2007       | 0.963 (0.318, 2.913) | 2.25 |        |
| Parfenov et al. 2007  | 0.307 (0.031, 3.034) | 1.07 |        |
| Baum et al. 2006      | 1.310 (0.414, 4.139) | 1.78 |        |
| Pezzini et al. 2005   | 1.101 (0.068, 17.815)| 0.33|        |
| Cerrato et al. 2005   | 0.229 (0.009, 5.663) | 0.63 |        |
| Jin et al. 2004       | 1.539 (0.254, 9.342) | 0.69 |        |
| Duzenli et al. 2004   | 0.769 (0.068, 8.700) | 0.55 |        |
| Souza et al. 2003     | 0.159 (0.008, 3.370) | 0.97 |        |
| Karttunen et al. 2002 | 2.481 (0.150, 41.118)| 0.20|        |
| Morrison et al. 2002  | 1.459 (0.824, 2.584) | 6.46 |        |
| MacLeod et al. 2001   | 0.913 (0.300, 2.780) | 2.29 |        |
| Chowdhury et al. 2001 | 0.439 (0.018, 10.878)| 0.46|        |
| Frikke-Schmidt et al. 2001 | 1.224 (0.788, 1.901)| 11.78|        |
| Catto et al. 2000     | 0.605 (0.216, 1.698) | 3.15 |        |
| Kokubo et al. 2000    | 1.484 (0.312, 7.060) | 0.81 |        |
| Peng et al. 1999      | 1.145 (0.070, 18.748)| 0.33|        |
| Ji et al. 1998        | 0.902 (0.196, 4.150) | 1.24 |        |
| Margaglione et al. 1998| 0.413 (0.023, 7.324) | 0.73|        |
| Kessler et al. 1997   | 0.941 (0.281, 3.154) | 1.92 |        |
| Hachinski et al. 1996 | 1.213 (0.074, 19.915)| 0.31|        |
| Couderc et al. 1993   | 0.680 (0.037, 12.474)| 0.45|        |
| Konialis et al. 2016  | 6.086 (0.311, 118.953)| 0.19|        |
| Fayed et al. 2009     | 9.545 (1.021, 89.223)| 0.23|        |
| Stankovic et al. 2004 | 0.347 (0.019, 6.197) | 0.83 |        |
| Pedro-Botet et al. 1992| 0.255 (0.012, 5.423) | 0.76|        |
| Artieda et al. 2008   | 1.491 (0.092, 24.088)| 0.28|        |
| Overall (I-squared = 12.0%, p = 0.254) | 1.233 (1.056, 1.440) | 100.00 |        |

(a)

**Figure 2:** Continued.
| Study ID           | OR (95% CI)                          | Weight |
|-------------------|--------------------------------------|--------|
| Wu et al. 2020    | 1.642 (1.256, 2.145)                 | 3.37   |
| Gelfand et al. 2013 | 2.063 (0.444, 9.580)            | 0.69   |
| Coen Herak et al. 2017 | 1.252 (0.520, 3.018)         | 1.53   |
| Jin et al. 2004   | 2.426 (1.405, 4.189)                 | 2.43   |
| Luo et al. 2015   | 0.968 (0.721, 1.300)                 | 3.29   |
| Pedro-Botet et al. 1992 | 2.556 (1.201, 5.437)        | 1.82   |
| Baum et al. 2006  | 1.075 (0.644, 1.793)                 | 2.54   |
| Yan et al. 2015   | 2.506 (1.630, 3.853)                 | 2.82   |
| Chatzistefanidis et al. 2014 | 1.459 (0.953, 2.233)   | 2.84   |
| Atadzhanov et al. 2013 | 0.799 (0.270, 2.368)         | 1.16   |
| Zhao et al. 2017  | 1.401 (0.981, 2.000)                 | 3.08   |
| Bakery et al. 2010 | 0.535 (0.184, 1.555)             | 1.19   |
| Tamam et al. 2009 | 1.250 (0.226, 6.902)                | 0.57   |
| Tascilar et al. 2009 | 0.889 (0.321, 2.459)            | 1.27   |
| Wang et al. 2009  | 2.950 (1.893, 4.599)                 | 2.78   |
| Lai et al. 2007   | 1.698 (0.954, 3.022)                 | 2.33   |
| Peng et al. 1999  | 2.720 (1.104, 6.703)                 | 1.49   |
| Karttunen et al. 2002 | 1.152 (0.520, 2.552)         | 1.72   |
| Duzenli et al. 2004 | 0.085 (0.011, 0.660)             | 0.42   |
| Frikkke-Schmidt et al. 2001 | 1.139 (0.957, 1.356)   | 3.63   |
| Pezzini et al. 2005 | 1.992 (1.101, 3.603)            | 2.28   |
| Cerrato et al. 2005 | 0.520 (0.306, 0.884)            | 2.48   |
| Das et al. 2016   | 1.074 (0.804, 1.435)                 | 3.31   |
| Kang and Lee. 2006 | 2.353 (1.302, 4.251)            | 2.28   |
| Slowik et al. 2003 | 0.693 (0.254, 1.894)            | 1.29   |
| Kessler et al. 1997 | 1.313 (0.820, 2.100)            | 2.69   |
| Wei et al. 2015   | 0.722 (0.480, 1.085)                 | 2.90   |
| Chowdhury et al. 2001 | 1.182 (0.660, 2.118)           | 2.31   |
| MacLeod et al. 2001 | 0.695 (0.454, 1.064)            | 2.84   |
| Fayad et al. 2009 | 15.000 (1.679, 134.025)            | 0.37   |
| Catto et al. 2010 | 0.883 (0.621, 1.255)                | 3.10   |
| Margagliano et al. 1998 | 1.919 (1.125, 3.273)         | 2.47   |
| Kokubo et al. 2000 | 0.970 (0.644, 1.461)            | 2.90   |
| Saidi et al. 2009 | 3.096 (2.048, 4.681)                | 2.88   |
| Brewin et al. 2020 | 1.366 (0.613, 3.045)            | 1.71   |
| Gao et al. 2006   | 2.311 (0.836, 6.392)                 | 1.27   |
| Souza et al. 2003 | 0.398 (0.161, 0.980)                | 1.49   |
| Hachinski et al. 1996 | 1.617 (0.785, 3.332)            | 1.90   |
| Couderc et al. 1993 | 0.692 (0.340, 1.409)            | 1.93   |
| Qian et al. 2012  | 1.475 (0.558, 3.902)                 | 1.35   |
| Konalis et al. 2016 | 2.118 (1.129, 3.973)            | 2.17   |
| Morrison et al. 2002 | 1.227 (0.938, 1.604)           | 3.37   |
| Stankovic et al. 2004 | 2.013 (1.059, 3.826)           | 2.13   |
| Koopal et al. 2016 | 0.927 (0.693, 1.240)            | 3.30   |
| Fekih-Mrissa et al. 2014 | 13.129 (0.672, 256.598) | 0.21   |
| Parfenov et al. 2007 | 1.381 (0.723, 2.637)            | 2.12   |
| Ji et al. 1998    | 2.491 (1.232, 5.037)                | 1.95   |
| Overall (I-squared = 68.9%, p = 0.000) | 1.340 (1.165, 1.542) | 100.00 |

**NOTE:** Weights are from random effects analysis.

**Figure 2:** Continued.
| Study ID | OR (95% CI) | Weight |
|---------|-------------|---------|
| Wu et al. 2020 | 2.789 (1.076, 7.228) | 3.07 |
| Zhao et al. 2017 | 8.438 (1.050, 67.815) | 0.53 |
| Coen Herak et al. 2017 | 0.492 (0.020, 12.314) | 0.66 |
| Das et al. 2016 | 1.012 (0.450, 2.277) | 6.38 |
| Atadzhanov et al. 2013 | 0.213 (0.011, 3.999) | 1.71 |
| Luo et al. 2015 | 0.948 (0.341, 2.632) | 4.16 |
| Wei et al. 2015 | 4.164 (0.851, 20.376) | 0.97 |
| Gelfand et al. 2013 | 11.000 (1.264, 95.692) | 0.17 |
| Parfenov et al. 2007 | 1.841 (0.325, 10.432) | 1.09 |
| Koopal et al. 2016 | 0.890 (0.429, 1.849) | 8.77 |
| Tamam et al. 2009 | 0.500 (0.030, 8.331) | 0.71 |
| Yan et al. 2015 | 2.156 (1.354, 3.433) | 14.05 |
| Balcerzyk et al. 2010 | 3.923 (0.424, 36.305) | 0.53 |
| Tas cleric et al. 2009 | 3.111 (0.611, 15.850) | 1.05 |
| Wang et al. 2009 | 4.849 (1.325, 17.754) | 1.35 |
| Lai et al. 2007 | 0.161 (0.006, 3.998) | 1.10 |
| Chatzistefanidis et al. 2014 | 2.449 (0.445, 13.494) | 0.98 |
| Kang and Lee. 2006 | 0.339 (0.014, 8.390) | 0.81 |
| Baum et al. 2006 | 5.239 (0.580, 47.339) | 0.47 |
| Pezzini et al. 2005 | 3.301 (0.133, 81.891) | 0.26 |
| Cerrato et al. 2005 | 0.687 (0.169, 2.787) | 2.55 |
| Jin et al. 2004 | 1.539 (0.254, 9.342) | 1.07 |
| Du eneli et al. 2004 | 0.511 (0.020, 12.785) | 0.64 |
| Slowik et al. 2003 | 1.206 (0.047, 30.768) | 0.38 |
| Souza et al. 2003 | 2.390 (0.096, 59.531) | 0.30 |
| Karttunen et al. 2002 | 0.351 (0.018, 7.016) | 1.07 |
| Morrison et al. 2002 | 1.604 (0.840, 3.065) | 7.31 |
| MacLeod et al. 2001 | 0.782 (0.155, 3.939) | 1.81 |
| Chowdhury et al. 2001 | 1.319 (0.183, 9.504) | 0.93 |
| Catto et al. 2000 | 0.883 (0.315, 2.470) | 4.17 |
| Kokubo et al. 2000 | 0.457 (0.059, 3.518) | 2.03 |
| Peng et al. 1999 | 2.291 (0.020, 25.959) | 0.50 |
| Ji et al. 1998 | 8.409 (0.428, 165.235) | 0.24 |
| Margaglione et al. 1998 | 37.424 (4.426, 316.422) | 0.15 |
| Frikke-Schmidt et al. 2001 | 0.871 (0.527, 1.439) | 18.95 |
| Saidi et al. 2009 | 7.076 (3.274, 15.291) | 2.72 |
| Hachinski et al. 1996 | 3.638 (0.366, 36.140) | 0.48 |
| Cou derc et al. 1993 | 1.077 (0.130, 8.938) | 0.88 |
| Qian et al. 2012 | 4.634 (0.257, 83.566) | 0.39 |
| Koniallis et al. 2016 | 6.086 (0.311, 118.953) | 0.29 |
| Fay et al. 2009 | 22.913 (1.196, 438.832) | 0.18 |
| Stankovic et al. 2004 | 3.504 (0.567, 21.664) | 0.52 |
| Pedro-Rotet et al. 1992 | 2.556 (0.611, 10.689) | 1.35 |
| Fekih-Missa et al. 2014 | 37.000 (1.004, 1364.036) | 0.04 |
| Brewin et al. 2020 | 4.157 (1.307, 13.220) | 1.21 |
| Kessler et al. 1997 | 3.951 (0.807, 19.351) | 1.00 |
| Overall (I-squared = 38.9%, p = 0.004) | 1.833 (1.542, 2.179) | 100.00 |

Figure 2: Continued.
Figure 2: Continued.
| Study ID | OR (95% CI) | % Weight |
|----------|-------------|----------|
| Hachinski et al. 1996 | 1.584 (0.811, 3.092) | 1.81 |
| Gelfand et al. 2013 | 3.500 (1.057, 11.586) | 0.91 |
| Wu et al. 2020 | 1.823 (1.426, 2.331) | 2.93 |
| Atadzhanov et al. 2013 | 0.914 (0.371, 2.253) | 1.33 |
| Zhao et al. 2017 | 1.463 (1.051, 2.035) | 2.72 |
| Balcerzyk et al. 2010 | 0.793 (0.319, 1.974) | 1.31 |
| Coen Herak et al. 2017 | 1.331 (0.584, 3.033) | 1.47 |
| Wang et al. 2009 | 2.503 (1.828, 3.429) | 2.76 |
| Koopal et al. 2016 | 0.927 (0.713, 1.206) | 2.89 |
| Yan et al. 2015 | 1.856 (1.414, 2.436) | 2.87 |
| Wei et al. 2015 | 0.598 (0.424, 0.843) | 2.69 |
| Peng et al. 1999 | 2.588 (1.146, 5.844) | 1.49 |
| Chatzistefanidis et al. 2014 | 1.263 (0.852, 1.874) | 2.55 |
| Gao et al. 2006 | 2.341 (0.852, 6.430) | 1.15 |
| Tasclar et al. 2009 | 0.944 (0.453, 1.966) | 1.66 |
| Tamam et al. 2009 | 0.912 (0.252, 3.303) | 0.82 |
| Cerrato et al. 2005 | 0.525 (0.320, 0.862) | 2.26 |
| Parfenov et al. 2007 | 1.509 (0.835, 2.730) | 2.00 |
| Duzenli et al. 2004 | 0.167 (0.038, 0.736) | 0.66 |
| Kang and Lee. 2006 | 2.171 (1.221, 3.859) | 2.04 |
| Das et al. 2016 | 1.090 (0.831, 1.429) | 2.87 |
| Jin et al. 2004 | 2.324 (1.397, 3.865) | 2.22 |
| Baum et al. 2006 | 1.204 (0.762, 1.901) | 2.37 |
| Souza et al. 2003 | 0.418 (0.179, 0.981) | 1.42 |
| Pezzini et al. 2005 | 2.010 (1.132, 3.571) | 2.05 |
| Slowik et al. 2003 | 0.737 (0.274, 1.982) | 1.18 |
| Lai et al. 2007 | 1.489 (0.886, 2.501) | 2.20 |
| Couderc et al. 1993 | 0.697 (0.355, 1.370) | 1.79 |
| Karttunen et al. 2002 | 1.050 (0.492, 2.243) | 1.60 |
| Morrison et al. 2002 | 1.293 (1.019, 1.641) | 2.95 |
| MacLeod et al. 2001 | 0.701 (0.473, 1.041) | 2.54 |
| Stankovic et al. 2004 | 2.129 (1.169, 3.875) | 1.98 |
| Chowdhury et al. 2001 | 1.162 (0.663, 2.034) | 2.08 |
| Ji et al. 1998 | 2.188 (1.157, 4.136) | 1.89 |
| Frikke-Schmidt et al. 2001 | 1.152 (0.982, 1.351) | 3.11 |
| Margaglione et al. 1998 | 2.093 (1.287, 3.405) | 2.29 |
| Catto et al. 2000 | 0.879 (0.636, 1.214) | 2.74 |
| Konialis et al. 2016 | 2.595 (1.404, 4.795) | 1.94 |
| Kokubo et al. 2000 | 0.883 (0.599, 1.304) | 2.56 |
| Pedro-Botet et al. 1992 | 2.144 (1.107, 4.152) | 1.83 |
| Luo et al. 2015 | 1.024 (0.780, 1.344) | 2.87 |
| Fayet et al. 2009 | 16.714 (3.378, 82.690) | 0.58 |
| Kessler et al. 1997 | 1.282 (0.836, 1.966) | 2.45 |
| Qian et al. 2012 | 1.759 (0.684, 4.525) | 1.25 |
| Brewin et al. 2020 | 2.307 (1.218, 4.368) | 1.88 |
| Fekih-Mrissa et al. 2014 | 20.879 (1.102, 395.483) | 0.20 |
| Li et al. 2016 | 1.739 (0.995, 3.038) | 2.09 |
| Saidi et al. 2009 | 3.123 (2.195, 4.444) | 2.66 |
| Artieda et al. 2008 | 1.282 (0.730, 2.253) | 2.07 |
| Schneider et al. 2005 | 2.107 (1.157, 3.837) | 1.98 |
| Overall (I-squared = 74.9%, p = 0.000) | 1.377 (1.203, 1.576) | 100.00 |

NOTE: Weights are from random effects analysis

Figure 2: Continued.
| Study ID  | OR (95% CI) | %  | Weight |
|----------|-------------|----|--------|
| Catto et al. 2000 | 1.458 (0.348, 6.112) | 2.88 |  |
| Peng et al. 1999 | 2.000 (0.051, 78.250) | 0.37 |  |
| Wu et al. 2020 | 1.806 (0.552, 5.908) | 3.86 |  |
| Zhao et al. 2017 | 9.143 (0.905, 92.398) | 0.54 |  |
| Coen Herak et al. 2017 | 0.067 (0.001, 5.749) | 1.39 |  |
| Das et al. 2016 | 0.667 (0.149, 2.979) | 3.93 |  |
| Wei et al. 2015 | 4.109 (0.776, 21.760) | 1.45 |  |
| Koopal et al. 2016 | 0.706 (0.256, 1.946) | 8.15 |  |
| Wang et al. 2009 | 2.506 (0.658, 9.540) | 2.91 |  |
| Luo et al. 2015 | 0.538 (0.141, 2.063) | 5.37 |  |
| Yan et al. 2015 | 3.175 (1.701, 5.924) | 10.27 |  |
| Atadzhanov et al. 2013 | 0.113 (0.005, 2.539) | 2.94 |  |
| Chatzistefanidis et al. 2014 | 5.333 (0.618, 45.991) | 0.66 |  |
| Gelfand et al. 2013 | 1.500 (0.106, 21.312) | 0.83 |  |
| Tamam et al. 2009 | 0.500 (0.013, 19.562) | 0.74 |  |
| Tascilar et al. 2009 | 8.167 (1.027, 64.936) | 0.59 |  |
| Lai et al. 2007 | 0.175 (0.006, 5.041) | 1.63 |  |
| Parfenov et al. 2007 | 6.000 (0.354, 101.568) | 0.37 |  |
| Baum et al. 2006 | 4.000 (0.340, 47.112) | 0.66 |  |
| Pezzini et al. 2005 | 3000 (0.060, 151.190) | 0.28 |  |
| Cerrato et al. 2005 | 3.000 (0.095, 95.170) | 0.38 |  |
| Jin et al. 2004 | 1.000 (0.080, 12.557) | 1.11 |  |
| Duzenli et al. 2004 | 0.556 (0.013, 24.513) | 0.70 |  |
| Souza et al. 2003 | 15.000 (0.182, 1236.183) | 0.09 |  |
| Karttunen et al. 2002 | 0.143 (0.003, 5.946) | 1.39 |  |
| Morrison et al. 2002 | 1.100 (0.478, 2.529) | 9.78 |  |
| Kessler et al. 1997 | 4.200 (0.586, 30.095) | 0.93 |  |
| MacLeod et al. 2001 | 8.157 (0.124, 5.944) | 2.05 |  |
| Saidi et al. 2009 | 3.136 (1.273, 7.723) | 5.10 |  |
| Frikke-Schmidt et al. 2001 | 0.712 (0.371, 1.366) | 20.07 |  |
| Kokubo et al. 2000 | 0.308 (0.024, 3.968) | 2.01 |  |
| Ji et al. 1998 | 9.000 (0.340, 238.210) | 0.27 |  |
| Margaglione et al. 1998 | 65.000 (2.239, 1887.351) | 0.09 |  |
| Hachinski et al. 1996 | 3.000 (0.084, 107.447) | 0.31 |  |
| Couderc et al. 1993 | 2.200 (0.075, 64.904) | 0.46 |  |
| Fayed et al. 2009 | 3.400 (0.120, 96.700) | 0.39 |  |
| Stankovic et al. 2004 | 10.714 (0.399, 287.828) | 0.23 |  |
| Pedro-Botet et al. 1992 | 9.286 (0.342, 252.450) | 0.25 |  |
| Brewin et al. 2020 | 0.825 (0.211, 3.219) | 4.25 |  |
| Chowdhury et al. 2001 | 3.000 (0.078, 115.338) | 0.33 |  |
| Overall (I-squared = 23.8%, p = 0.092) | 1.625 (1.281, 2.060) | 100.00 |  |

Figure 2: Continued.
3.2.4. APOE ε2 Carrier Comparisons. In the genetic model of ε2 carriers vs. non-ε2 carriers, there was no association with the IS risk (pooled OR = 0.956, 95% CI 0.841–1.086, P = 0.49) (Table 2).

3.2.5. APOE ε4 Homozygosis versus APOE ε4 Heterozygote Comparisons. Given the above, the APOE ε4 mutation was linked to IS risk. To identify whether there is a dose-response relationship between the ε4 allele and IS or not, we implemented the comparisons between the ε4/ε4 genotype and ε4 heterozygotes (ε2/ε4 or ε3/ε4 genotype). Compared with the ε2/ε4 and ε3/ε4 genotypes, the IS risk ORs for ε4/ε4 genotypes were 1.625 (95% CI, 1.281–2.060, P < 0.0001) and 1.301 (95% CI, 1.077–1.571, 0.83).

Figure 2: (a–g) Forest plots of the relationships between APOE gene polymorphisms in all studies included. (a) Forest plot of ε2/ε4 vs. ε3/ε3 comparison. (b) Forest plot of ε3/ε4 vs. ε3/ε3 comparison. (c) Forest plot of APOE ε4/ε4 vs. the ε3/ε3 genotype. (d) Forest plot of the APOE ε4 allele vs. ε3 allele. (e) Forest plot of APOE ε4 carriers vs. non-ε4 carriers. (f) Forest plot of APOE ε4/ε4 vs. ε2/ε4. (g) Forest plot of APOE ε4/ε4 vs. ε3/ε4.

Table 2: Dose-response relationship between APOE ε4 genotype and IS risk. The ORs were calculated using the fixed-effects model and adjusted for age and sex. The forest plots show the results of this analysis.
| Study ID          | OR (95% CI)          | % Weight | Weight |
|------------------|----------------------|----------|--------|
| Zhao et al. 2017 | 0.252 (0.014, 4.411) | 4.89     | 4.89   |
| Das et al. 2016  | 1.346 (0.299, 6.062) | 4.40     | 4.40   |
| Luo et al. 2015  | 2.081 (0.746, 5.806) | 7.21     | 7.21   |
| Chatzistefanidis et al. 2014 | 0.322 (0.040, 2.603) | 6.79     | 6.79   |
| Souza et al. 2003| 0.159 (0.008, 3.370) | 4.24     | 4.24   |
| Lai et al. 2007  | 1.216 (0.356, 4.150) | 7.20     | 7.20   |
| Cerrato et al. 2005 | 0.597 (0.024, 14.810)| 1.65     | 1.65   |
| Tascilar et al. 2009 | 0.381 (0.092, 1.573) | 10.29   | 10.29 |
| Kokubo et al. 2000 | 3.199 (0.388, 26.352)| 0.92     | 0.92   |
| Kessler et al. 1997 | 2.547 (0.685, 9.471) | 3.67     | 3.67   |
| Pedro-Botet et al. 1992 | 0.567 (0.026, 12.235)| 1.96     | 1.96   |
| Subtotal (I-squared = 7.7%, p = 0.370) | 0.978 (0.607, 1.576)| 53.21    | 53.21  |
| Zhao et al. 2017 | 1.003 (0.056, 17.955)| 1.43     | 1.43   |
| Das et al. 2016  | 1.448 (0.076, 27.462)| 0.98     | 0.98   |
| Luo et al. 2015  | 1.760 (0.214, 14.440)| 1.62     | 1.62   |
| Cerrato et al. 2005 | 1.577 (0.063, 39.561)| 0.80     | 0.80   |
| Kokubo et al. 2000 | 5.688 (0.675, 47.892)| 0.53     | 0.53   |
| Kessler et al. 1997 | 0.776 (0.090, 6.670) | 3.17     | 3.17   |
| Subtotal (I-squared = 0.0%, p = 0.850) | 1.458 (0.534, 3.980)| 8.53     | 8.53   |
| Zhao et al. 2017 | 0.832 (0.218, 3.175) | 7.56     | 7.56   |
| Das et al. 2016  | 4.360 (0.779, 24.408)| 1.26     | 1.26   |
| Luo et al. 2015  | 0.880 (0.185, 4.187) | 5.41     | 5.41   |
| Kokubo et al. 2000 | 0.810 (0.046, 14.208)| 1.77     | 1.77   |
| Lai et al. 2007  | 0.734 (0.190, 2.832) | 7.67     | 7.67   |
| Cerrato et al. 2005 | 1.006 (0.040, 25.081)| 1.15     | 1.15   |
| Chatzistefanidis et al. 2014 | 0.599 (0.125, 2.864)| 7.13     | 7.13   |
| Kessler et al. 1997 | 0.622 (0.034, 11.488)| 2.13     | 2.13   |
| Pedro-Botet et al. 1992 | 0.897 (0.041, 19.627)| 1.37     | 1.37   |
| Wen et al. 2006  | 1.447 (0.233, 8.995) | 2.83     | 2.83   |
| Subtotal (I-squared = 0.0%, p = 0.921) | 0.932 (0.526, 1.652)| 38.26    | 38.26  |
| Overall (I-squared = 0.0%, p = 0.891) | 1.002 (0.709, 1.414)| 100.00   | 100.00 |

Figure 3: Continued.
| Study ID | OR (95% CI) | Weight |
|----------|-------------|---------|
| LAA      |             |         |
| Zhao et al. 2017 | 1.143 (0.818, 2.440) | 3.91 |
| Das et al. 2016 | 0.556 (0.369, 0.838) | 4.54 |
| Luo et al. 2015 | 1.136 (0.793, 1.628) | 4.77 |
| Cerrato et al. 2005 | 0.485 (0.230, 1.023) | 3.07 |
| Tascler et al. 2009 | 0.889 (0.321, 2.459) | 2.19 |
| Lai et al. 2007 | 1.226 (0.619, 2.431) | 3.31 |
| Kang and Lee. 2006 | 2.216 (1.145, 4.290) | 3.41 |
| Gao et al. 2006 | 1.533 (0.329, 7.147) | 1.22 |
| Chatzistefanidis et al. 2014 | 1.479 (0.877, 2.494) | 4.02 |
| Slowik et al. 2003 | 0.438 (0.115, 1.663) | 1.51 |
| Kokubo et al. 2000 | 2.154 (1.173, 3.956) | 3.63 |
| Souza et al. 2003 | 0.398 (0.161, 0.980) | 2.52 |
| Kessler et al. 1997 | 1.777 (0.940, 3.360) | 3.51 |
| Pedro-Botet et al. 1992 | 3.096 (1.276, 7.512) | 2.57 |
| **Subtotal (I-squared = 68.3%, p = 0.000)** | **1.154 (0.841, 1.584)** | **44.16** |
| CE       |             |         |
| Zhao et al. 2017 | 1.089 (0.362, 3.278) | 1.98 |
| Das et al. 2016 | 3.157 (1.823, 5.467) | 3.90 |
| Luo et al. 2015 | 0.997 (0.453, 2.194) | 2.90 |
| Cerrato et al. 2005 | 0.259 (0.059, 1.127) | 1.31 |
| Kokubo et al. 2000 | 1.351 (0.530, 3.448) | 2.42 |
| Kessler et al. 1997 | 0.975 (0.432, 2.199) | 2.82 |
| **Subtotal (I-squared = 67.4%, p = 0.009)** | **1.175 (0.627, 2.203)** | **15.32** |
| SAD      |             |         |
| Zhao et al. 2017 | 1.178 (0.748, 1.857) | 4.33 |
| Das et al. 2016 | 1.929 (1.144, 3.254) | 4.02 |
| Luo et al. 2015 | 0.872 (0.555, 1.370) | 4.35 |
| Chatzistefanidis et al. 2014 | 1.581 (0.956, 2.613) | 4.11 |
| Lai et al. 2007 | 2.125 (1.144, 3.949) | 3.58 |
| Kang and Lee. 2006 | 2.560 (1.252, 5.233) | 3.19 |
| Gao et al. 2006 | 3.040 (0.765, 12.086) | 1.44 |
| Cerrato et al. 2005 | 0.903 (0.430, 1.898) | 3.08 |
| Slowik et al. 2003 | 0.905 (0.306, 2.682) | 2.01 |
| Kokubo et al. 2000 | 0.687 (0.345, 1.367) | 3.29 |
| Kessler et al. 1997 | 1.540 (0.627, 3.785) | 2.53 |
| Pedro-Botet et al. 1992 | 2.123 (0.695, 6.487) | 1.94 |
| Wen et al. 2006 | 1.592 (0.673, 3.767) | 2.65 |
| **Subtotal (I-squared = 36.2%, p = 0.093)** | **1.392 (1.097, 1.767)** | **40.52** |
| **Overall (I-squared = 59.1%, p = 0.000)** | **1.270 (1.049, 1.538)** | **100.00** |

**NOTE:** Weights are from random effects analysis.

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Figure 3: Continued.
### Disease Markers

**Study ID**
- Das et al. 2016
- Zhao et al. 2017
- Luo et al. 2015
- Chatzistefanidis et al. 2014
- Tascliar et al. 2009
- Lai et al. 2007
- Kang and Lee 2006
- Cerrato et al. 2005
- Slowik et al. 2003
- Souza et al. 2003
- Kokubo et al. 2000
- Kessler et al. 1997
- Pedro-Botet et al. 1992

| OR (95% CI) | Weight |
|------------|--------|
| 0.748 (0.260, 2.147) | 17.48 |
| 12.918 (1.327, 125.717) | 0.78 |
| 1.189 (0.354, 3.988) | 9.73 |
| 2.574 (0.358, 18.504) | 2.31 |
| 3.111 (0.611, 15.850) | 4.00 |
| 0.338 (0.014, 8.416) | 3.07 |
| 0.560 (0.023, 13.917) | 2.31 |
| 0.449 (0.049, 4.078) | 5.85 |
| 2.633 (0.102, 68.073) | 1.07 |
| 2.390 (0.096, 59.531) | 1.15 |
| 0.934 (0.054, 16.054) | 2.11 |
| 1.910 (0.169, 21.616) | 1.70 |
| 1.917 (0.302, 12.171) | 3.07 |
| 1.367 (0.886, 2.236) | 54.63 |

**CE**
- Zhao et al. 2017
- Das et al. 2016
- Luo et al. 2015
- Cerrato et al. 2005
- Kokubo et al. 2000
- Kessler et al. 1997

| OR (95% CI) | Weight |
|------------|--------|
| 5.682 (0.225, 143.647) | 0.35 |
| 2.202 (0.473, 10.253) | 3.42 |
| 0.818 (0.046, 14.443) | 2.34 |
| 0.526 (0.028, 9.998) | 3.19 |
| 1.641 (0.094, 28.649) | 1.22 |
| 2.328 (0.205, 26.462) | 1.45 |

**Subtotal (I-squared = 0.0%, p = 0.896)**

| OR (95% CI) | Weight |
|------------|--------|
| 1.543 (0.591, 4.029) | 11.98 |

**SAD**
- Das et al. 2016
- Zhao et al. 2017
- Luo et al. 2015
- Chatzistefanidis et al. 2014
- Lai et al. 2007
- Kang and Lee 2006
- Cerrato et al. 2005
- Kokubo et al. 2000
- Kessler et al. 1997
- Pedro-Botet et al. 1992
- Wen et al. 2006

| OR (95% CI) | Weight |
|------------|--------|
| 0.727 (0.093, 5.706) | 5.02 |
| 11.091 (1.286, 95.680) | 1.28 |
| 0.880 (0.185, 4.187) | 7.28 |
| 2.397 (0.334, 17.218) | 2.43 |
| 0.306 (0.012, 7.623) | 3.22 |
| 0.848 (0.034, 21.167) | 1.75 |
| 0.335 (0.018, 6.335) | 4.56 |
| 1.068 (0.137, 8.303) | 3.59 |
| 8.278 (1.098, 62.405) | 0.88 |
| 4.600 (0.845, 25.052) | 2.09 |
| 4.341 (0.383, 49.255) | 1.29 |
| 1.809 (1.030, 3.175) | 33.39 |
| 1.536 (1.086, 2.171) | 100.00 |

| Weight |
|--------|
| 0.0696 |
| 144    |

**Overall (I-squared = 0.0%, p = 0.802)**

**Figure 3: Continued.**
**Figure 3:** Continued.
### Table 1: Meta-analysis results

| Study ID          | OR (95% CI)         | % Weight |
|-------------------|---------------------|----------|
| **LAA**           |                     |          |
| Zhao et al. 2017  | 1.526 (0.915, 2.546) | 3.96     |
| Das et al. 2016   | 0.629 (0.432, 0.917) | 4.80     |
| Luo et al. 2015   | 1.240 (0.891, 1.727) | 5.08     |
| Chatzistefanidis et al. 2014 | 1.301 (0.798, 2.122) | 4.09     |
| Tascilar et al. 2009 | 0.944 (0.453, 1.966) | 2.82     |
| Lai et al. 2007   | 1.187 (0.643, 2.191) | 3.40     |
| Kang and Lee. 2006 | 2.126 (1.116, 4.052) | 3.24     |
| Gao et al. 2006   | 1.795 (0.390, 8.270) | 0.99     |
| Subtotal (I-squared = 63.4%, p = 0.001) |                     |          |
| **CE**            |                     |          |
| Zhao et al. 2017  | 1.051 (0.353, 3.126) | 1.69     |
| Das et al. 2016   | 2.349 (1.422, 3.879) | 1.02     |
| Luo et al. 2015   | 1.000 (0.477, 2.096) | 2.80     |
| Cerrato et al. 2005 | 0.246 (0.057, 1.662) | 1.06     |
| Kokubo et al. 2000 | 1.181 (0.502, 2.776) | 2.36     |
| Kessler et al. 1997 | 0.894 (0.429, 1.861) | 2.83     |
| Subtotal (I-squared = 59.2%, p = 0.031) |                     |          |
| **SAD**           |                     |          |
| Zhao et al. 2017  | 1.629 (0.838, 3.192) | 4.55     |
| Das et al. 2016   | 1.938 (1.182, 3.179) | 4.06     |
| Luo et al. 2015   | 0.847 (0.556, 1.288) | 4.52     |
| Chatzistefanidis et al. 2014 | 1.324 (0.831, 2.109) | 4.24     |
| Lai et al. 2007   | 1.758 (1.000, 2.091) | 3.66     |
| Kang and Lee. 2006 | 2.232 (1.115, 4.666) | 3.00     |
| Gao et al. 2006   | 2.885 (0.732, 11.375) | 1.18     |
| Cerrato et al. 2005 | 0.749 (0.364, 1.542) | 2.88     |
| Slowik et al. 2003 | 0.887 (0.302, 2.608) | 1.72     |
| Kokubo et al. 2000 | 0.707 (0.367, 1.364) | 3.18     |
| Kessler et al. 1997 | 1.422 (0.638, 3.167) | 2.55     |
| Pedro-Botet el al. 1992 | 2.158 (0.840, 5.541) | 2.07     |
| Wen et al. 2006   | 1.746 (0.823, 3.704) | 2.75     |
| Subtotal (I-squared = 37.1%, p = 0.087) |                     |          |
| **Overall (I-squared = 53.2%, p = 0.000)** |                     |          |
| NOTE: Weights are from random effects analysis
Figure 3: Continued.
3.3. Main Results of the Relationship between APOE Gene and Three IS Subtypes. We further investigated on the correlation of APOE gene polymorphisms and risks of IS subtypes by making comparisons in five genetic models, with a particular focus on the APOE e4 mutation. Subgroup analyses showed that APOE e4 mutation significantly increased SAD risk (e4 allele vs. e3 allele: pooled OR = 1.318, 95% CI, 1.073-1.618, \( P = 0.01 \) (Figure 3(d)); e3/e4 vs e3/e3: pooled OR = 1.392, 95% CI, 1.097-1.767, \( P = 0.01 \) (Figure 3(b)); e4/e4 vs. e3/e3: pooled OR = 1.809, 95%, CI 1.030-3.175, \( P = 0.04 \) (Figure 3(c)); and APOE e4 carriers vs. non-APOE e4 carriers: pooled OR = 1.329, 95% CI, 1.064-1.661, \( P = 0.01 \) (Figure 3(e))). But genotype e2/e4 did not increase the risk of SAD onset (Figure 3(a)). The result of APOE e4 homozygosis versus e4 heterozygote comparisons (e4/e4 vs. e2/e4 and e4/e4 vs. e3/e4) was a matter of concern: APOE e4 mutation could not cause a cumulative effect in generating higher risk of SAD onset, as showed in Figures 3(f) and 3(g).

3.4. Sensitivity Analysis. Sensitivity analysis was performed by removing studies one by one to check the effect of the individual study on overall ORs. No single study influenced on the pooled ORs and 95% CIs in all genetic model comparisons as our data showed (supplementary material table 4).
3.5. Publication Bias. We carried out publication bias analysis by using funnel plots as qualitative description and Egger’s regression tests as quantitative outcome. Funnel plots of all genetic model comparisons did not exhibit apparent asymmetry (several funnel plots were showed in supplementary material figure 1 and 2). In addition to subtype analysis of \( \varepsilon2/\varepsilon2 \) vs. \( \varepsilon3/\varepsilon3 \), all the Egger’s regression test outcomes indicated that there existed no evident publication bias with all \( P \) values exceeding 0.1 (supplementary material table 5). The above results showed that publication bias of our meta-analysis was not significant.

3.6. Regression Analysis. Meta-regression analysis was then performed to explore sources of heterogeneity as shown in supplementary material table 5, considering the year of publication, region, sample size, genotyping method, HWE, NOS score, and source of control. However, the \( P \) value of each factor affecting overall heterogeneity was not statistically significant in comparisons of \( \varepsilon3/\varepsilon4 \) vs. \( \varepsilon3/\varepsilon3, \varepsilon4 \) vs. non-\( \varepsilon4, \varepsilon2 \) vs. non-\( \varepsilon2, \varepsilon4 \) allele vs. \( \varepsilon3 \) allele, and \( \varepsilon2 \) allele vs. \( \varepsilon3 \) allele (supplementary material figure 3). Heterogeneity sources were unascertainable.

3.7. The Result of Trial Sequential Analysis (TSA). The RIS was 8901 samples and the sample size of our meta-analysis reached it. Moreover, the cumulative z-curve crossed the trial sequential monitoring boundary before reaching the RIS as showed in Figure 4. The result of TSA guaranteed the stability of our meta-analysis results. Our sample size was proved to be enough for evaluating the relationship between APOE polymorphisms and IS risk.

4. Discussion

Recently, scholars explored more how gene polymorphisms were contributing to the occurrence and prognosis of diseases. And several previous publications had well explored how gene polymorphisms related to diseases onset and potential mechanisms [74, 75]. As a heterogeneous multifactorial disorder, ischemic stroke could be regulated by certain gene synthesis and specific gene products. The genes involved in the pathological process of stroke are also worth of attention. Apolipoprotein E has been proven to affect atherosclerosis, neurodegeneration, and the process of nerve damage repair. That is why we explored the relationship between APOE gene polymorphisms and ischemic stroke risk.

APOE is a 299-amino acid protein encoded by the APOE gene of three common polymorphisms, \( \varepsilon2, \varepsilon3, \) and \( \varepsilon4 \). The correlation of APOE gene polymorphisms and the risk of cerebral vascular and degenerative diseases have been investigated a lot, especially in Alzheimer’s disease (AD) and cerebral amyloid angiopathy (CAA) [76]. APOE \( \varepsilon4 \) is associated with increased risk for AD whereas APOE \( \varepsilon2 \) is associated with decreased risk [77]. Mirza et al. performed a meta-analysis to find that greater WMH volume was associated with worse performance on all cognitive domains in APOE \( \varepsilon4 \) carriers only in AD [78]. Charidimou et al. proved that
the APOE e2 allele might be associated with the pathophysiology and severity of cortical superficial siderosis in CAA [79]. As to IS, there existed quite many researches with inconsistent conclusions. Besides method differences, ethnic difference and unclarified pathophysiological mechanisms are probable reasons of the inconsistency.

In a meta-analysis in 1999, McCarron et al. found that the e4 allele and carriers were more frequent among patients with ischemic cerebrovascular disease, compared with control subjects (27% versus 18%; odds ratio, 1.73; 95% CI, 1.34-2.23; \( P < 0.0001 \)) [13]. In another meta-analysis based on Chinese population, the e4 allele is associated with an increased risk of developing cerebral infarction, in which the adjusted risk estimate for the e4 allele versus e3 allele was significant (\( OR = 2.00, 95\% \ CI \: 1.59-2.53, \: P < 0.0001 \)) [14]. Our estimates seemed to be coinciding with the above ones. Compared with the e3 allele, the e4 allele showed a higher risk of IS. Compared with e3/e3, both e4 heterozygote (e2/e4, e3/e4) and e4 homoyzgote (e4/e4) exhibited a significant correlation with an increased risk of IS. Notably, OR in e4 homozyzgote (e4/e4 vs. e3/3: 1.833 (95% CI 1.542-2.179)) was higher than those in e4 heterozygotes (e2/e4 vs. e3/3: 1.233 (95% CI 1.056-1.440) and e3/e4 vs. e3/3: 1.340 (95% CI 1.165-1.542)), which implied that the e4 allele might possess a cumulative effect. Then, we performed comparisons between e4/e4 and e2/e4 or e3/e4; there existed significant differences between e4 homozygote and e4 heterozygote. The OR between e4/e4 and e2/e4 was 1.625 (95% CI 1.281-2.060, \( \: P < 0.0001 \)); the OR between e4/e4 and e3/e4 was 1.301 (95% CI 1.077-1.571, \( \: P = 0.01 \)), giving a hint that e4 homozygote might bring a higher risk of IS than e4 heterozygotes.

There are tremendous researches and discussions focusing on the pathogenicity of e4. An Indian research reported that VLDL and triglycerides levels were found to be significantly associated with e2/e4 and e3/e4 genotypes; the e4 allele exerted a higher influence than the e3 allele in plasma cholesterol levels [22]. As a lipid transport protein, APOE3 and APOE2 preferentially bind to the smaller, more phospholipid-enriched high-density lipoproteins (HDL), while APOE4 preferentially binds to the larger, triglyceride-rich very low-density lipoproteins (VLDL). Miyata and Smith demonstrated an antioxidant activity in the order APOE2 > E3 > E4, and other researchers also reported similar results that APOE4 was associated with increased oxidative stress [25, 80], which might play a role in atherosclerosis and lead to increased risk of ischemic vascular diseases. Besides the above reasons, APOE4 was proved to be neurotoxic by assuming an abnormal conformation (the unique domain interaction between Arg-61 and Glu-255) which was highly susceptible to neuron specific proteolysis and generating neurotoxic fragments that escaped the secretory pathway and entered the cytosol [81]. Totally, from pathophysiological mechanisms to clinical research results, it seems that APOE4 is indeed related to a higher risk of IS, compared with other isoforms, both in e4 heterozygote and homozygous. e2 allele appears to be unclear and controversial in stroke [13]. In a meta-analysis of Martinez-González et al., compared with e3/e3, APOE ε2 was associated with intracerebral hemorrhage (OR = 1.32; 95% CI, 1.01-1.74); meanwhile, APOE ε2 was more related to lobar hemorrhage than deep hemorrhage [82]. As to the association of IS with APOE based on previous investigation, it is uncertain. Our estimates showed that both e2/e2 and e2/e3 genotypes exhibited no significant effects on IS risk, compared with e3/e3. Also, no differences were found in comparisons of e2 allele vs. e3 allele and e2 vs. non-e2 carriers. This result remained consistent with another meta-analysis in 2013 [14]. Interestingly, in subtype analysis, e2/e2 displayed significances in the CE group (OR = 4.290; 95% CI, 1.917-9.600; \( \: P < 0.0001 \)) and SAD group (OR = 1.803; 95% CI, 1.037-3.134; \( \: P = 0.04 \)). The largest meta-analysis of the APOE genotype with IS showed a positive linear association of increasing risk when ordered from e2/e2, e2/e3, e2/e4, e3/e3, e3/e4, and e4/e4 in European ancestry population [83]. The conclusion might explain why APOE4 brings a higher risk of IS but could not clarify if the CE and SAD subgroups in comparison of e2/e2 with e3/e3 show significances. It is well known that all patients with type III hyperlipidemia (dysbetalipoproteinemia) were APOE2 homozygous, whereas most e2/e2 subjects (>90%) were normolipidemic or even hypolipidemic, owing to reductions in LDL or HDL or both. Therefore, the APOE ε2 allele has both increased and decreased risks for atherosclerosis, which induced a comprehensive and undetermined result [84].

As to our subtype analyses, all LAA groups showed no significant difference among comparisons, which raised a question why isoforms of APOE, a lipid transport protein, seemed not to be related with IS caused by large artery atherosclerosis. Besides lipid metabolism and atherosclerosis, there might exist some other pathways underlying the relationships between APOE and risk of IS. Our estimates displayed that APOE isoforms were associated to risk of IS especially in the SAD subgroup. Hypertension was known to be an independent risk factor of SAD. Atherosclerosis, dyslipidemias, and hypertension have a complex interaction, and the causations with APOE need further investigation.

Our meta-analysis has several limitations. First, just as the abovementioned, heterogeneity between studies remains undeterminable. Second, results of our meta-analysis based on case control studies cannot provide a causal relationship, but only an association. Third, age variable and ethnicity can affect the APOE frequencies in a population; we cannot obtain sufficient related information to perform further subdivided subgroup analyses. Fourth, other pathogenic factors about IS, a multifactorial disease, such as plasma lipid levels, hypertension, life-style, BMI, and gene-environment interactions, were unachievable. Fifth, the controls in accessible studies were not strictly defined; some were selected from healthy populations and others were from nonstroke people. The expected genotype distribution in controls was not in accordance with HWE in seven studies. Population selection in control groups failed to avoid certain diseases which might have a relation with the APOE gene, such as dyslipidemia, hypertension, other vascular diseases, and diabetes. Sixth, the case groups were not selected by a prospective process and the design of case control studies often caused abnormal gene frequency.
5. Conclusions

In conclusion, our meta-analysis provides rational evidence that APOE ε4 mutation is a genetic risk factor for IS. Prospective studies of a large sample size, which concerns gene-gene and gene-environment interactions, should be carried out in the future to reach a more comprehensive outcome about the association of APOE gene polymorphisms and IS. What is more, future researches should be designed to elucidate the mechanism by which APOE ε4 mutation adds the risk of IS.

Data Availability

Data presented within the paper and the supplementary materials contributed to the findings in our study. They are all are available from our corresponding author for reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

Authors’ Contributions

The conceptualization was done by S.-Y. Q., K. S., Y.-H.C., and X.C.; the methodology was done by D.-S. T., D.-J. P., and C. Q.; K. S., Y.-H.C., H.-H. Y, and X.C. took care of the software; meta-analysis was done by D.-S. T., D.-J. P., C. Q., S.-Y. Q., K. S., and X.C.; writing—original draft preparation—was done by S.-Y. Q., K. S., Y.-H.C., and H.-H. Y.; writing—review and editing—was done by D.-S. T., D.-J. P., and C. Q. All authors have read and agreed to the published version of the manuscript. Su-Ya Qiao and Ke Shang contributed equally to this work.

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Supplementary Materials

Supplementary material Table 1: fifteen of the included studies provide data about different subtypes of IS: LAA, SAD, and CE. Supplementary material Table 2: Newcastle-Ottawa Scale (NOS) score of included studies. Supplementary material Table 3: PRISMA list of our meta-analysis. Supplementary material Table 4: sensitivity analysis of the association between ApoE gene polymorphisms and IS. Supplementary material Table 5: publication bias and heterogeneity of our meta-analysis. Supplementary material Figure 1: funnel plots for studies included in Figures 2A–G. Supplementary material Figure 2: funnel plots for studies included in Figures 3A–G. Supplementary material Figure 3: results of meta-regression. (Supplementary Materials)

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