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# Polymorphisms of apolipoprotein E and aneurysmal subarachnoid haemorrhage: A meta-analysis

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ABSTRACT

Subarachnoid haemorrhage (SAH) is characterised by bleeding in the subarachnoid space in the brain. There are various polymorphisms in genes which are associated with this disease. We performed a systematic meta- analysis to investigate the relationship of *APOE* polymorphism on aSAH. A comprehensive literature search was done in the Pubmed database, Science Direct, Cochrane library and Google Scholar. The OR and 95% CI were evaluated for the gene and aSAH association using fixed and random effect models. Publication bias was assessed using Begg's funnel plot and Egger's regression test. All statistical evaluations were done using the software Review Manager 5.0 and Comprehensive Meta Analysis v2.2.023. A total of 9 studies were assessed on *APOE* polymorphism (1100 Cases, 2732 Control). Meta analysis results showed significant association in ε2/ ε2 versus ε3/ε3, ε2 versus ε3 genetic models and ε2 allele frequency. In subgroup analysis statistically significant association was observed in Asians in the genetic models ε2/ ε2 versus ε3/ε3, ε2/ε3 versus ε3/ε3, ε2 versus ε3 and also in ε2 allele frequency. However, in Caucasian population only ε2/ε2 versus ε3/ε3 genetic model showed significant association between *APOE* and risk of aSAH. In this meta-analysis study, the ε2/ε2 genotype is associated with increased risk of aSAH.



**Contents**



## 1. Introduction

Subarachnoid haemorrhage (SAH) is a pathological condition characterised by bleeding into the subarachnoid space which is the area between the arachnoid membrane and the pia mater surrounding

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the brain (Suarez et al., 2006).SAH is mainly caused by three reasons, rupture of a cerebral arterial aneurysm, an arterial-venous malformation, or head trauma (Ruigrok et al., 2005). An aneurysm is a localized bulge in the wall of an artery (Raya and Diringer, 2014). Risk factors of aneurysmal subarachnoid haemorrhage (aSAH) include hypertension, smoking, female gender and heavy alcohol intake (Feigin et al., 2005). In addition, studies have also shown the importance of genetic factors caused by polymorphisms in the pathogenesis of aSAH (Woo et al., 2002). Though there is surgical and medical progress, the treatment of aSAH remains unpredictable and cause high rate of mortality, especially in WFNS grade III and IV (Al Shahi et al., 2006).

The association between *apolipoprotein E* (*APOE*) and neurocognitive outcomes after SAH has been investigated in many studies on different population. *APOE* protein combines with lipids to form lipoprotein particles which are involved in the metabolism and transport of lipids in the central nervous system (Mahley and Rall, 2000. In humans, *APOE* gene is located on the long arm of chromosome 19 at position q13.2. *APOE* is a polymorphic gene with three major alleles (ε2, ε3 ε4) coding for three isoforms of proteins (E2, E3, E4), which differ on the 112th and 158th amino acid position with cysteine and arginine interchange (Csajbok et al., 2015). The three allelic variants of *APOE* are defined by two SNPs, rs7412 and rs429358 (Yuan et al., 2015). The six possible genotypes of *APOE* were derived from the combination of polymorphisms rs7412 and rs429358. The C allele at both SNPs constitutes ε4 allele and T allele at both SNPs constitutes ε2 allele. The C allele at rs7412 and T allele at rs429358 identify ε3 allele (Radmanesh et al., 2014).

The presence of the *ε4* allele is known to be associated with increased risk of developing Alzheimer's disease (Strittmatter et al., 1994), poor outcomes after traumatic brain injury (Teasdale et al., 2005) and intracerebral haemorrhage (Alberts et al., 1995). *APOE* polymorphism could also contribute to the progression of atherosclerosis by affecting the serum levels of cholesterol and triglyceride (Chalouhi et al., 2012). Association between the *APOE* polymorphism and risk of aSAH has been investigated in several studies but the results were conflicting. To resolve this question, a systematic meta-analysis was performed to verify the association between *APOE* gene polymorphisms and the risk of aSAH.

## 2. Materials and methods

### 2.1. Search strategy

We searched all published studies in the Pubmed database, Science Direct, Cochrane library and Google Scholar using the following combination of keywords: "*Apolipoprotein E*" or "*APOE*", "*APOE* Polymorphism", "Subarachnoid haemorrhage", "*APOE* polymorphism and aSAH", "Single nucleotide polymorphism", "Allele Variation/genotype". The data source for this study was obtained from literature published up to March2016.

### 2.2. Inclusion and exclusion criteria

The inclusion criteria for the selected articles were as follows: (1) case–control study, (2) reports on the association between *APOE* polymorphisms and aSAH (3) studies with full text articles, and (4) the studies which confirm aSAH by lumbar puncture/CT scanning/conventional angiography. The exclusion criteria for the selected articles were as follows: case reports, systemic reviews, data on genotype frequency was not reported , clinical trials, meta analysis, in vitro studies, articles which are not written in English.

### 2.3. Data extraction

The following information was extracted from each included article: first author, year of publication, country, ethnicity, source of controls,

**Table 1**
Characteristics of all eligible studies and distribution of *Apo E* genotype of cases and controls included in the meta-analysis.

| First Author(Year) | Country | Ethnicity | Source | Genotyping Method | No: of Cases/Control | Genotype for Cases/Controls | | | | | | Allele for Cases/Controls | | | HW-E[a] | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | ε2/ε2 | ε2/ε3 | ε2/ε4 | ε3/ε3 | ε3/ε4 | ε4/ε4 | ε2 | ε3 | ε4 | *P* Value | |
| McCarron et al. (1998) | UK | Caucasians | HB[c] | PCR_RFLP | 96/406 | 2/4 | 48/222 | 4/12 | 8/45 | 5/13 | 29/ 110 | 56/66 | 69/ 325 | 67/ 245 | 0.76 | McCarron et al. (1998) |
| Kokubo et al. (2000) | Japan | Asian | HB[c] | PCR_RFLP | 37/1126 | 1/11 | 2/73 | 0/8 | 21/ 819 | 11/ 202 | 2/13 | 4/103 | 55/1913 | 15/ 236 | / | Kokubo et al. (2000) |
| Tang et al. (2003) | China | Asian | HB[c] | PCR-RFLP | 104/123 | 0/2 | 17/13 | 2/2 | 69/89 | 15/17 | 1/0 | 19/19 | 170/208 | 19/19 | 0.29 | Tang et al. (2003) |
| Yamada et al. (2004) | Japan | Asian | HB[c] | PCR_RFLP | 100/100 | NA[b] | NA[b] | NA[b] | NA[b] | NA[b] | NA[b] | 8/7 | 171/ 164 | 21/29 | NA[b] | Yamada et al. (2004) |
| Kaushal et al. (2006) | USA | Caucasians | HB[c] | Taq man assay | 107/202 | 0/1 | 10/32 | 1/2 | 61/97 | 20/46 | 2/6 | 11/36 | 152/ 272 | 25/60 | 0.22 | Kaushal et al. (2006) |
| Mineharu et al. (2006) | Japan | Asian | HB[c] | PCR_RFLP | 362/332 | NA[b] | NA[b] | NA[b] | NA[b] | NA[b] | NA[b] | 13/13 | 44/38 | 305/ 280 | NA[b] | Mineharu et al. (2006) |
| Fontanella et al. (2007) | Italy | Caucasians | HB[c] | PCR-RFLP | 146/222 | 6/1 | 17/28 | 1/0 | 107/161 | 15/31 | 0/1 | 30/30 | 246/ 381 | 16/33 | 0.43 | Fontanella et al. (2007) |
| Csajbok et al. (2015) | Sweden | Caucasians | HB[c] | Taq man assay | 148/221 | NA[b] | NA[b] | NA[b] | NA[b] | NA[b] | NA[b] | NA[b] | NA[b] | 46/68 | NA[b] | Csajbok et al. (2015) |
| Liu et al. (2016) | China | Asian | HB[c] | PCR-RFLP | 150/150 | 9/1 | 35/21 | 4/7 | 76/80 | 25/31 | 1/10 | 57/30 | 212/ 212 | 31/58 | 0.08 | Liu et al. (2016) |

[a] Hardy–Weinberg equilibrium.
[b] Data not given.
[c] Hospital Based.

genotyping method, total numbers of aSAH cases and controls and distribution of genotypes and alleles in aSAH cases and controls (Table 1).

### 2.4. Statistical analysis

The data were evaluated using the software Review Manager (Version 5.0, Cochrane Collaboration) and Comprehensive Meta Analysis v2.2.023. Genotype ε3/ε3 was assigned as the reference group in our study. For *APOE* gene the genotype frequencies for ε2/ε2 versus ε3/ε3, ε2/ε3 versus ε3/ε3, ε2/ε4 versus ε3/ε3, ε3/ε4 versus ε3/ε3, ε4/ε4 versus ε3/ε3, allele ε2 versus allele ε3, and allele ε4 versus allele ε3 were analyzed. The allele frequencies were also analyzed for ε2, ε3 and ε4. The genetic association and risk of aSAH was calculated using odds ratio (OR) and 95% confidence interval (CI). The test for heterogeneity was calculated by Cochran's Q test and $I^2$ statistics. For Q test, $P$ value <0.10 was considered as significant for heterogeneity among the studies. The $I^2$ value represents the percentage of total variation across the studies due to heterogeneity. $I^2$ values of 25%, 50% and 75% were considered as low, moderate, and high heterogeneity. When there was significant heterogeneity, we use random effect model to pool the results. Odds ratios were calculated with either fixed effects model (MantelHaenszel method) or random effects model (DerSimonian-Laird method) according to the heterogeneity (Mantel and William, 1959). Fixed effect model was applied when there was no heterogeneity; otherwise random effect model was applied (DerSimonian and Laird, 1986). The publication bias was assessed by Begg's funnel plot, and Egger's regression tests for ≥3 publications (Begg and Mazumdar, 1994; Egger et al., 1997). The Hardy-Weinberg equilibrium was determined based on the control genotyping results. Hardy-Weinberg equilibrium was analyzed with the online software (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl). ε3/ε3 genotype is the wild type homozygote genotype for *APOE* gene, while ε4/ε4 and ε2/ε2 are the rare homozygous genotypes. The heterozygous genotypes for *APOE* gene are ε2/ε3, ε2/ε4 and ε3/ε4.

## 3. Results

### 3.1. Study characteristics

A total of 809 studies were reviewed based on our selection strategy. The step wise selection process in this meta analysis is mentioned in [Fig. 1]. A total of 9 potentially relevant studies (Csajbok et al., 2015; Fontanella et al., 2007; Kaushal et al., 2006; Kokubo et al., 2000; Liu et al., 2016; McCarron and Nicoll, 1998; Mineharu et al., 2006; Tang et al., 2003; Yamada et al., 2004) met the inclusion criteria. Studies were carried out in China, Japan, Italy, UK, USA, Sweden, Netherlands and Spain. Genotyping methods included in the studies were PCR-RFLP and Taqman assay. Detailed characteristics of the studies including allele and genotype frequencies of *APOE* in this meta analysis are mentioned in Table 1. The HWE was assessed on the genotype distribution of the controls in all included studies ($P_{HWE} > 0.05$).

### 3.2. Association of APOE polymorphism and aSAH

Nine studies were identified that evaluated the association of *APOE* polymorphism with the risk of aSAH. OR with 95% CI for the seven genetic models and allele frequencies for ε2, ε3, ε4 was assessed. The association between *APOE* polymorphisms and the risk of aSAH was statistically significant in the genetic models of ε2/ε2 versus ε3/ε3 [OR = 3.30, 95% CI = 1.48–7.37, $P = 0.004$] (Fig. 2) and ε2 versus ε3 [OR = 1.26, 95% CI = 1.00–1.59, $P = 0.05$] (Fig. 2). The association was also seen statistically significant in ε2 allele frequency [OR = 1.28, 95% CI = 1.02–1.60, $P = 0.03$] (Fig. 2). In our meta analysis ε4 allele frequency [OR = 0.90, 95% CI = 0.69–1.18, $P = 0.44$] (Fig. 2) showed no significant association with the risk on aSAH. The results of meta analysis are summarized in Table 2.

Furthermore in the subgroup analysis the association between *APOE* polymorphisms and the risk of aSAH were statistically significant in Asians in the genetic models ε2/ε2 versus ε3/ε3 [OR = 3.09, 95% CI = 1.01–9.47, $P = 0.05$] (Fig. 3), ε2/ε3 versus ε3/ε3 [OR = 1.65, 95% CI = 1.04–2.61, $P = 0.03$] (Fig. 3) and ε2 versus ε3 [OR = 1.46, 95% CI = 1.06–2.02, $P = 0.02$] (Fig. 3). The ε2 allele frequency also showed statistical significance in the Asian population [OR = 1.51, 95% CI = 1.11–2.06 $P = 0.009$] (Fig. 3). In Caucasian population only ε2/ε2 versus ε3/ε3 genetic model showed significant association [OR = 3.55, 95% CI = 1.12–11.3, $P = 0.03$] (Fig. 3). No other genetic models in Asians and Caucasians showed a significant association between the *APOE* and aSAH. The results of subgroup analysis are summarized in Table 2.

### 3.3. Publication bias

Publication bias was assessed by Begg's funnel plot and Egger's regression test for *APOE*. The genetic models of ε2/ε2 versus ε3/ε3 [$P = 0.21$] (Supplementary Fig. 1), ε2 allele versus ε3 allele [$P = 0.66$] (Supplementary Fig. 1), ε2/ε3 versus ε3/ε3 [$P = 0.88$], ε4/ε4 versus ε3/ε3[$P = 0.91$], ε3/ε4 versus ε3/ε3 [$P = 0.74$], ε2/ε4 versus ε3/ε3 [$P = 0.87$], ε2 allele versus ε3 allele [$P = 0.66$] (Supplementary Fig. 1),

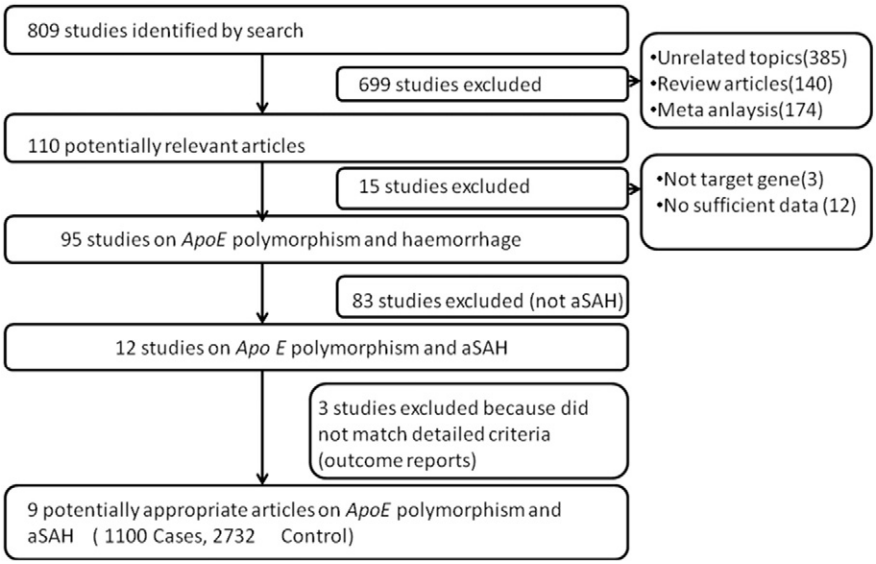
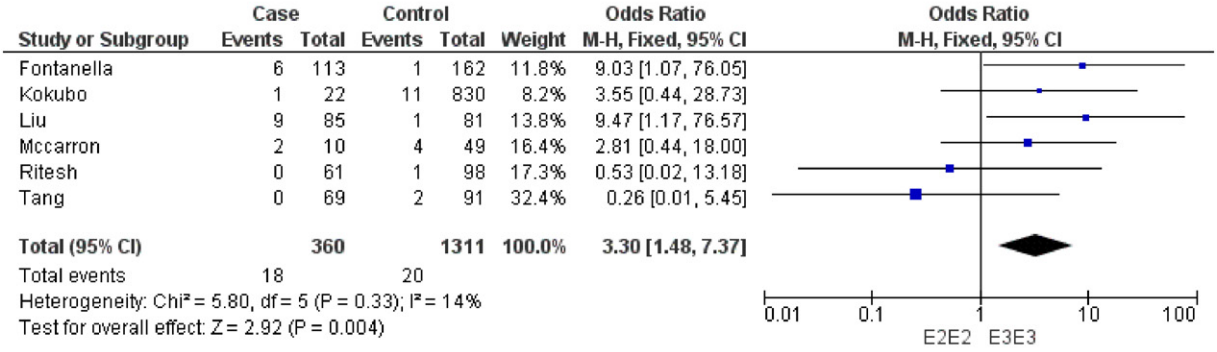
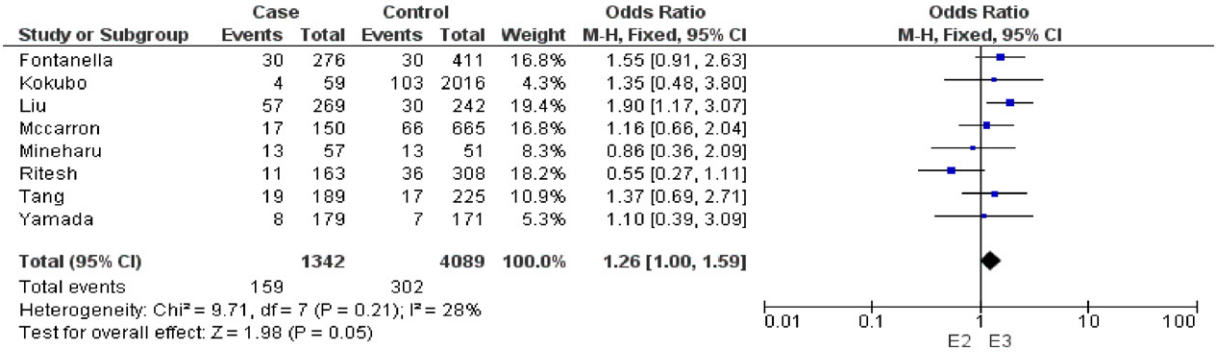



**Fig. 1.** Flow diagram of study selection.

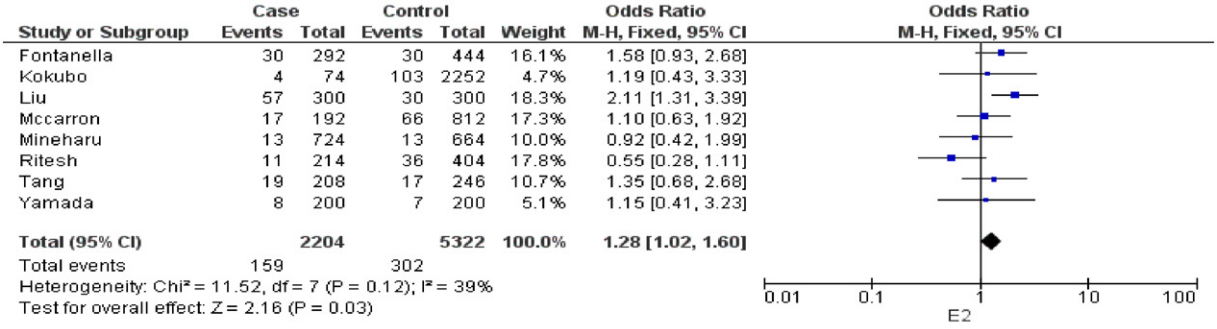
(A) ε2/ε2 versus ε3/ ε3




(B) ε2 versus ε3




(C) ε2 allele frequency




(D) ε4 allele frequency

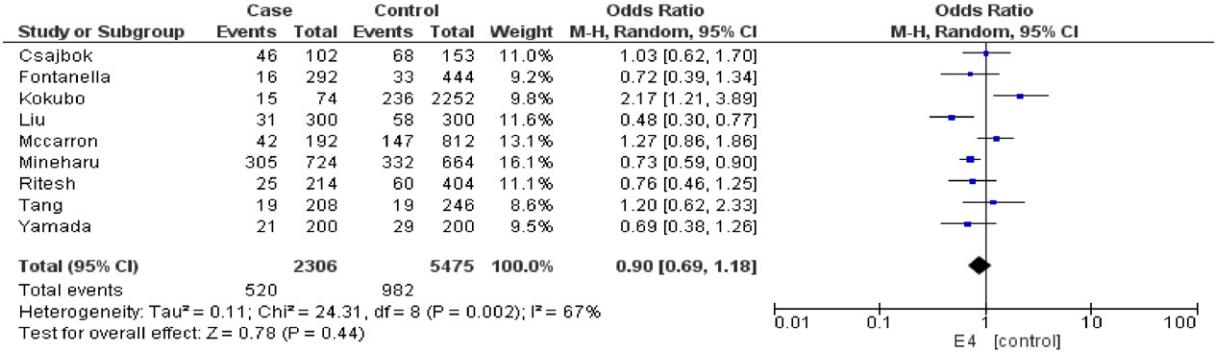



**Fig. 2.** Forest plots of OR with 95% CI for the association of *APOE* polymorphisms and aSAH risk in the genetic model (A) ε2/ε2 versus ε3/ε3 (B) ε2 versus ε3 (C) ε2 allele frequency (D) ε4 allele frequency.

**Table 2**
Summary of data of genes and their polymorphisms investigated and their odds ratio with 95%.

| SL no | Gene | Overall/subgroup | Fixed effects OR (95%CI) | Random effects OR (95% CI) | $I^2$ for heterogeneity (%) | *P* value for heterogeneity | *P* value for Z test |
|---|---|---|---|---|---|---|---|
| | ApoE | | | | | | |
| 1 | ε2 ε2 vs ε3 ε3 | All | 3.30 [1.48.7.37] | 3.14 [1.15.8.58] | 14 | 0.33 | **0.004** |
| | | Asian | 3.09 [1.01.9.47] | 2.71 [0.43.17.7] | 45 | 0.16 | **0.05** |
| | | Caucasian | 3.55 [1.12.11.3] | 3.25 [0.85.12.2] | 7 | 0.34 | **0.03** |
| 2 | ε2 ε3 vs ε3ε3 | All | 1.05 [0.78.1.43] | 1.05 [0.69.1.58] | 38 | 0.15 | 0.74 |
| | | Asian | 1.65 [1.04.2.61] | 1.65 [1.03.2.62] | 0 | 0.83 | **0.03** |
| | | Caucasian | 0.73 [0.48.1.12] | 0.74 [0.48.1.13] | 0 | 0.48 | 0.15 |
| 3 | ε2 ε4 vs ε3 ε3 | All | 1.20 [0.61.2.37] | 1.24 [0.62.2.45] | 0 | 0.76 | 0.60 |
| | | Asian | 0.82 [0.30.2.25] | 0.86 [0.31.2.33] | 0 | 0.64 | 0.70 |
| | | Caucasian | 1.71 [0.68.4.32] | 1.71 [0.67.4.37] | 0 | 0.69 | 0.25 |
| 4 | ε4 ε4 vs ε3 ε3 | All | 0.92 [0.52.1.62] | 1.05 [0.33.3.35] | **60** | **0.03** | 0.93 |
| | | Asian | 0.63 [0.23.1.75] | 1.31 [0.06.29.7] | **84** | **0.002** | 0.87 |
| | | Caucasian | 1.12 [0.55.2.26] | 1.13 [0.54.2.37] | 0 | 0.48 | 0.75 |
| 5 | ε3 ε4 vs ε3 ε3 | All | 1.00 [0.77.1.29] | 1.02 [0.74.1.39] | 30 | 0.21 | 0.98 |
| | | Asian | 1.17 [0.78.1.76] | 1.23 [0.72.2.11] | 43 | 0.17 | 0.44 |
| | | Caucasian | 0.89 [0.64.1.25] | 0.89 [0.61.1.29] | 18 | 0.30 | 0.51 |
| 6 | ε2 vs ε3 | All | 1.26 [1.00.1.59] | 1.23 [0.93.1.64] | 28 | 0.21 | **0.05** |
| | | Asian | 1.46 [1.06.2.02] | 1.46 [1.06.2.01] | 0 | 0.58 | **0.02** |
| | | Caucasian | 1.07 [0.77.1.49] | 1.03 [0.59.1.82] | **63** | **0.07** | 0.69 |
| 7 | ε4 vs ε3 | All | 0.95 [0.79.1.14] | 0.98 [0.71.1.35] | **67** | **0.004** | 0.89 |
| | | Asian | 0.93 [0.73.1.18] | 1.03 [0.62.1.72] | **77** | **0.002** | 0.91 |
| | | Caucasian | 0.97 [0.74.1.28] | 0.94 [0.64.1.39] | 46 | 0.16 | 0.75 |
| 8 | ε2 allele frequency | All | 1.28 [1.02.1.60] | 1.23 [0.91.1.67] | 39 | 0.12 | **0.03** |
| | | Asian | 1.51 [1.11.2.06] | 1.49 [1.09.2.06] | 2 | 0.40 | **0.009** |
| | | Caucasian | 1.06 [0.76.1.47] | 1.02 [0.58.1.81] | **64** | **0.06** | 0.73 |
| 9 | ε3 allele frequency | All | 0.94 [0.81.1.09] | 0.93 [0.78.1.11] | 27 | 0.21 | 0.42 |
| | | Asian | 0.94 [0.76.1.15] | 0.91 [0.69.1.20] | 43 | 0.15 | 0.53 |
| | | Caucasian | 0.95 [0.77.1.17] | 0.94 [0.74.1.20] | 21 | 0.28 | 0.62 |
| 10 | ε4 allele frequency | All | 0.83 [0.72.0.95] | 0.90 [0.69.1.18] | **67** | **0.002** | 0.44 |
| | | Asian | 0.76 [0.64.0.91] | 0.88 [0.56.1.38] | **78** | **0.001** | 0.58 |
| | | Caucasian | 0.98 [0.77.1.24] | 0.97 [0.74.1.27] | 19 | 0.30 | 0.84 |

*P* value less than 0.05 is represented as bold figures in the table.

ε4 allele versus ε3 allele [$P = 0.89$], ε2 allele frequency [$P = 0.58$] (Supplementary Fig. 1), ε3 allele frequency [$P = 0.23$] and ε4 allele frequency [$P = 0.93$] (Supplementary Fig. 1), showed no significant publication bias. No significant publication bias was seen in all genetic models.

### 3.4. Sensitivity analysis

Sensitivity analysis was conducted to determine the source of heterogeneity and also to find out the stability and reliability of the present study by sequentially omitting each individual studies. The results showed that none of the individual studies altered the pooled odds ratio of all genetic models (Supplementary Fig. 2). However after excluding the study done by Kokubo et al., and Liu et al., we found no evidence of heterogeneity in the genetic models such as ε4ε4 versus ε3ε3 ($P = 0.57$; $I^2 = 0\%$), ε4 versus ε3 ($P = 0.20$; $I^2 = 32\%$) and ε4 allele frequency ($P = 0.36$; $I^2 = 3\%$) in Asian population. In Caucasians after omitting the study done by Kaushal et al., no evidence of heterogeneity was observed in the genetic models such as ε2 versus ε3($P = 0.47$; $I^2 = 0\%$) and ε2 allele frequency ($P = 0.35$; $I^2 = 0\%$).

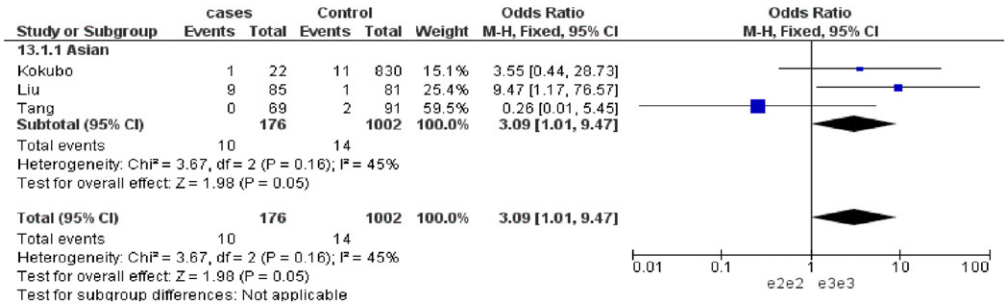
## 4. Discussion

aSAH is a critical clinical problem with less chances of patient recovery and survival even after surgical management and medication. Development of personalized therapy might show significant improvement in the management of this clinical problem. Discovery of the effect of gene polymorphisms in the course of treatment show hope in the field of personalized therapies. *APOE* polymorphism is one among them and it has been shown in various studies that it is associated with risk of aSAH in different populations. In order to make a conclusion about the role of this polymorphism with the risk of aSAH, we performed a meta-analysis using nine previously published case–control studies.

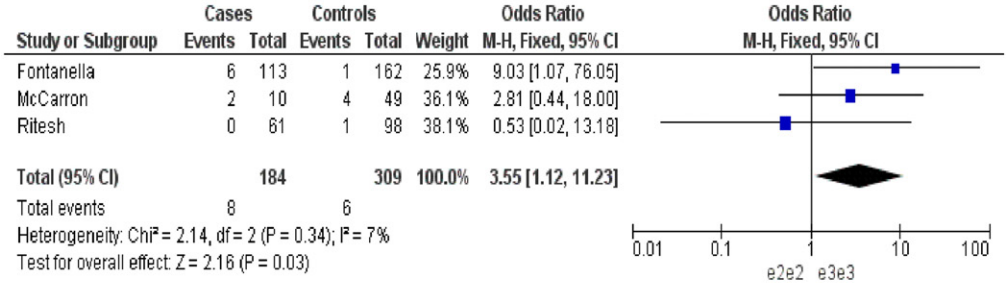
*APOE* protein is expressed mainly in endothelial cells of arterial walls both in normal and aneurysmal arteries (Rinkel, 2008). The *APOE* plays an important role in maintaining lipid homeostasis in central nervous system (Gong et al., 2002), neuronal repair (Buttini et al., 1999), maintaining synaptic plasticity (Nathan et al., 1994), neuronal migration and differentiation (Bellosta et al., 1995), mitochondrial damage due to oxidative stress (Gibson et al., 2000), cerebral glucose metabolism (Reiman et al., 2004). The *APOE* gene polymorphism is associated with the risk of developing many central nervous system disorders. *APOE* polymorphism has been recognized as a genetic risk factor for the development of Alzheimer's disease in different populations (Achouri-Rassas et al., 2015; Kumar et al., 2015; Zheng et al., 2016). In Chinese population, *APOE* polymorphism is associated with the risk of developing deep venous thrombosis (Zhu et al., 2014). Various meta-analysis data showed that *APOE* polymorphism is associated with risk of fronto-temporal lobar degeneration (Rubino et al., 2013), vascular dementia (Yin et al., 2012a, 2012b), hypertension (Stoumpos et al., 2013), multiple sclerosis (Yin et al., 2012a, 2012b), Myocardial Infarction (Xu et al., 2014) and cerebral infraction (Wang et al., 2013).

The exact molecular mechanism of *APOE* polymorphism and risk of aSAH still remains unclear. The presence of ε4 and ε2 alleles increases the risk of delayed ischemic neurologic deficit (DIND) for aSAH patients (Lanterna et al., 2005). Studies showed that individuals with *ε4* allele have increased deposition of amyloid β-protein (Aβ) in the brain parenchyma compared to individuals with other *APOE* polymorphism (Kaushal et al., 2006; Qiu et al., 2015). Proposed model for the formation of aneurysm suggests that atherosclerosis was a common pathological feature (Chalouhi et al., 2012). Compared with ε3 homozygote, ε4 allele and ε2 allele carriers have higher circulating low density lipoprotein cholesterol (Davignon, 2005) which suggests that ε4 and ε2 alleles may have more chances of developing atherosclerosis and thereby causing aSAH.

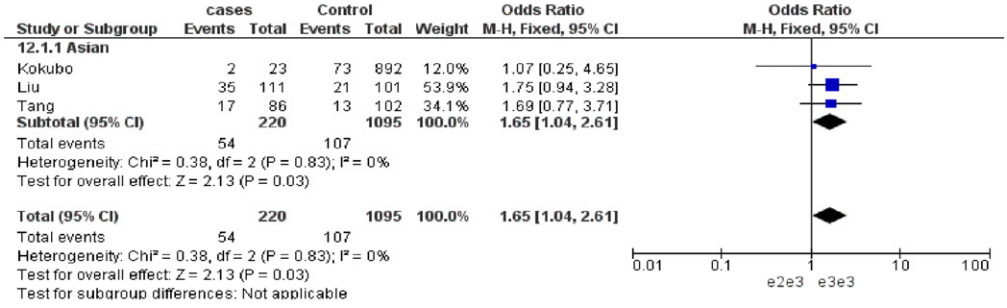
(A) ε2/ ε2 versus ε3/ ε3[Asian]



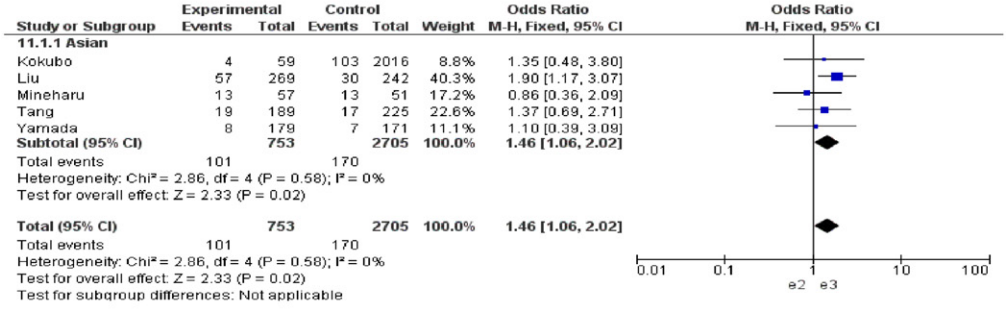

(B) ε2/ ε2 versus ε3/ ε3[Caucasian]




(C) ε2/ ε3 versus ε3/ ε3[Asian]




(D) ε2 versus ε3[Asian]




(E) ε2 allele frequency[Asian]

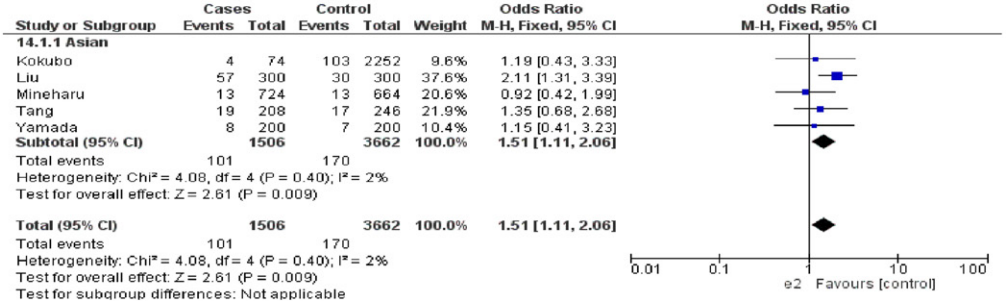



**Fig. 3.** Forest plots of OR with 95%CI in the subgroup analysis for the association of *APOE* polymorphisms and aSAH risk in the genetic model (A) ε2/ε2 versus ε3/ε3[Asian] (B) ε2/ε2 versus ε3/ε3[Caucasian] (C) ε2/ε3 versus ε3/ε3[Asian] (D) ε2 versus ε3[Asian] (E) ε2 allele frequency[Asian].

Previously three different meta analysis investigated the association of *APOE* polymorphism and outcome in patients with aSAH. Lanterna et al., conducted a meta analysis on eight case control studies to investigate the association of ε4 allele and negative outcome after SAH (Lanterna et al., 2007). The study also investigated the association between the ε4 allele and delayed ischemia which is a major complication of SAH. They found that the ε4 allele was associated with a higher risk of a negative outcome and delayed ischemia. Martinez-Gonzalez et al., conducted meta analysis on five case control studies, that showed association of ε4allele and negative outcome after aSAH (Martinez-Gonzalez and Sudlow, 2006). The third study conducted by Sudlow et al., on three case control studies showed that ε4 allele has a significant association with the risk of SAH (Sudlow et al., 2006). Among these three studies only Sudlow et al., showed the association of *APOE* polymorphism and risk of SAH while other two studies showed association with patient's outcome after SAH. These studies evaluated only ε4 allele and did not consider the other genetic models of *APOE*. In our research, we performed an updated meta analysis including all the seven genetic models and three allele frequency of *APOE* to establish the role of *APOE* polymorphism and risk of aSAH.

In our meta analysis ε4 allele showed no significant association between *APOE* polymorphism and the risk of aSAH as shown in previous studies. Similarly we did not find any correlation between *APOE* and aSAH in any other genetic models. However, our study showed significant association between *APOE* polymorphism and the risk of aSAH in the genetic model ε2/ε2 versus ε3/ε3 and ε2 versus ε3. The ε2 allele frequency also showed significant association between *APOE* polymorphism and risk of aSAH. In our study we found significant heterogeneity in three genetic models ε4ε4 versus ε3ε3, ε4 versus ε3 and ε4 allele frequency. Since the subjects in the study were included from different populations there was a possibility for genetic heterogeneity. To eliminate heterogeneity we conducted sub group analysis based on ethnicity and used random effect model to pool the results whenever significant heterogeneity was present. The results showed no heterogeneity in majority of genetic models except in the models such as ε4ε4 versus ε3ε3, ε4 versus ε3 and ε4 allele frequency in Asian population. In Caucasian population, ε2 versus ε3 model and ε2 allele frequency showed heterogeneity. The main factors that may lead to heterogeneity are sample size, diversity in study design, inclusion criteria, genotyping method and the genotype distribution of controls which were not in agreement with HWE. In addition, environmental exposure and diet can cause heterogeneity (Daly and Day, 2001; Ioannidis et al., 2008). Our sensitivity analysis suggested that the inclusion of studies conducted by Kokubo et al., and Liu et al., (Kokubo et al., 2000; Liu et al., 2016) contributed to heterogeneity in Asian population and Kaushal et al., (Kaushal et al., 2006) in Caucasian population.

Our study has few limitations that should be considered. Firstly, our meta-analysis included studies with accessible full-text articles in English only. Therefore, the missing of other eligible studies that were reported in other languages could lead to unavoidable publication bias in the result. Secondly, due to the limited sample size included in this study, it was hardly possible for us to perform other subgroup analysis. Despite these limitations our study has certain advantages. Firstly, the procedural issues in meta-analysis, such as heterogeneity, publication bias, and the stability of the results were well investigated. Secondly, our results were robust because the results of the sensitivity analysis were not altered and hence the conclusion remained the same.

In conclusion, the pooled data showed significant association between *APOE* genotype and risk of aSAH in ε2 allele not in ε4 allele. Only few studies have been conducted to address the association between *APOE* polymorphisms and the risk of aSAH. There are potential opportunities to perform further studies and prove the conclusions arrived at through the investigations.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mgene.2016.06.003.

## Conflict of interest

The authors have declared that no competing interests exist on the materials or methods used in this study and findings specified in this paper.

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