Meta-analysis of the association between Apolipoprotein E polymorphism and risks of myocardial infarction

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Abstract

Background: Myocardial infarction (MI) remains the leading cause of death and disability among cardiovascular diseases worldwide. Studies show that elevated low-density lipid protein cholesterol (LDL-C) levels confer the highest absolute risk of MI, and Apolipoprotein E (ApoE) is implicated in regulating levels of triglycerides (TGs), cholesterol, and LDL-C. Our study aimed to evaluate the association between APOE polymorphism and MI, and to provide evidence for the etiology of MI.

Methods: Case–control studies on the association between APOE polymorphisms and the risk of myocardial infarction were included by searching PubMed, Web of Science, and CNKI, and this meta-analysis was written in accordance with PRISMA guideline statement. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using either random-effects or fixed-effects models by R software.

Results: A total of 33 eligible articles involving 13,706 cases and 14,817 controls were finally selected. The pooled analysis based on the total eligible articles showed that the risk of MI was associated with ApoE epsilon 2 and epsilon 4 alleles. The results showed that patients with MI had a low frequency of the ε2 allele (OR 0.74, 95% CI 0.64–0.86) and a high frequency of the ε4 allele (OR 1.24, 95% CI 1.09–1.42).

Conclusions: APOE ε2-involved genotypes may be protective factors for MI; in contrast, ε4-involved genotypes (ε4/ε3 vs. ε3/ε3, and ε4/ε4 vs. ε3/ε3) may be risk factors for MI.

Keywords: Apolipoprotein E polymorphism, Myocardial infarction, Meta-analysis

Introduction

Myocardial infarction (MI) remains the leading cause of death and disability among cardiovascular diseases worldwide [1]. Blood lipid abnormalities are implicated in MI: elevated low-density lipid protein cholesterol (LDL-C) levels confer the highest absolute risk of MI [2]. Apolipoprotein E (ApoE) is implicated in regulating levels of triglycerides (TGs), cholesterol, and LDL-C [3]. Myocardial infarction is usually due to thrombotic occlusion of a coronary vessel caused by the rupture of a vulnerable plaque [4]. Ischemia induces severe ion disturbance in the myocardium [4]. Vulnerable plaques tend to have 30 – 50% stenosis, thin fibrous caps and contain more inflammatory cells such as lipid-laden macrophages [5]. Infiltrated phagocytes clear dead cells and matrix debris, activate anti-inflammatory pathways, and inhibit cytokine and chemokine signaling [4]. Activation of the renin–angiotensin–aldosterone system and release of
transforming growth factor-beta promotes the transformation of fibroblasts into myofibroblasts [4].

Epidemiological findings show that the impact of myocardial infarction on global health is significant, with more than one-third of deaths in developed countries [5]. Today, NSTEMI (non-ST-segment elevation myocardial infarction) accounts for 60–75% of all myocardial infarctions. In addition, both in-hospital and 1-year mortality from STEMI (ST-segment elevation myocardial infarction) has declined over the past two decades (5–6% and 7–18%, respectively) [5]. The prevalence of MINOCA (myocardial infarction with no obstructive coronary atherosclerosis) was 6% (95% CI 5.1–7%) [6], the median age of patients was 55 years (95% CI 51–59 years), and 40% were female. The 12-month mortality in MINOCA patients was 4.7% (95% CI 4.1–5.4%) [6]. The Framingham Heart Study’s 10-year follow-up data revealed that the incidence of MI was 12.9, 38.2, and 71.2 per 1000 in men and 2.2, 5.2, and 13.0 per 1000 in women in the age groups of 30–34, 35–44, and 45–54 years, respectively[7].

The study showed that, regardless of age, more women than men died within one year of the first acute myocardial infarction (AMI) (26% of women and 19% of men respectively) and more women than men died within 5 years of the first AMI (47% of women and 36% of men). At 5 and 10 years after AMI, women had a higher unadjusted mortality rate compared to men and had a 30% readmission rate within 30 days of the first hospitalization, partly due to differences in age, MI risk factors, clinical presentation, and treatment. Women also have a higher prevalence of heart failure and diabetes mellitus (DM) compared to men[8]. A meta-analysis has also shown that myocardial infarction is associated with genotype[9].

The exon 4 of APOE has two single nucleotide polymorphisms (SNPs) (rs7412 and rs429358). The two SNPs are used to define the three major alleles of APOE (ε2, ε3, and ε4). Allele ε3 possesses cysteines in the amino-acid-coding positions corresponding to rs7412 and rs429358, conferring APOE3 with arginine at residue 158 and cysteine on residue 112 [10]. ε2 arises from substitution rs7412C>T, and rs429358C>T results in ε4. Thus, APOE2 carries cysteine at residue 158 and 112, and APOE4 carries arginine on both positions [11]. Because allele ε3 is the most common in populations, this allele is used as “wild-type”. ε2 and ε4 are used as variants of APOE alleles [12]. The six APOE haplotypes (ε2ε2, ε2ε3, ε2ε4, ε3ε3, ε3ε4, and ε4ε4) are formed by combinations of these three alleles [13].

Associations of APOE polymorphism and MI risks have been investigated extensively [14–17]. In 2014, Xu H. et al. performed a meta-analysis, finding that the frequency of MI increases for ε4ε4 vs. ε3ε3 (OR1.59, 95% CI1.15–2.19, \( P = 0.005 \)); whereas, no significant association exists in ε2ε2 vs. ε3ε3 (OR0.73, 95% CI0.40–1.32, \( P = 0.29 \)) [18]. In contrast, a meta-analysis issued in 2015 revealed that, for ε2ε2 vs. ε3ε3, a decreased frequency of MI exists (OR0.40, 95% CI0.20–0.83, \( P = 0.00 \)), except in Caucasian and Asian populations, and no significant association exists in ε4ε4 vs. ε3ε3 (OR1.34, 95% CI0.91–1.98, \( P = 0.186 \)) in these populations [19].

Possible reasons for the above results are: (1) they had different inclusion and exclusion criteria: Xu H. et al’s study in 2014 did not consider cancer risk, but such studies were included in the 2015 article, further led to a large difference in the number of articles finally included in the study between the two: in 2014 (n = 33); in 2015 (n = 22); (2) the results of 2015 divided the ethnic group into three subgroups and found that Caucasians and Asians have different gene expression frequencies compared to other ethnic groups. But 2014 results only compared two subgroups of Caucasians and Asians. Thus, we conducted an up-to-date meta-analysis to resolve these conflicting results.

Materials and methods

Search strategy

According to the PRISMA guideline, we searched all articles published before May 1, 2021, from both English databases (PubMed, and Web of Science database) and Chinese databases (CNKI database) using the combination of keywords (“Apolipoprotein E” OR “ApoE” OR “APOE” AND “myocardial infarction” OR “MI” AND “polymorphism” OR “polymorphisms” OR “variants” OR “variant”). In addition, we searched related articles that had not been included in the initial search using Google (www.google.com).

Inclusion and exclusion criteria

Articles were included for further selection if they fulfilled the inclusion criteria: (1) articles issued in English or Chinese were performed under either hospital-based or population-based design; (2) evaluation of the association between APOE polymorphisms and MI was involved and the data can be extracted in articles; and (3) odds ratios (ORs) with 95% confidence intervals (CIs) were evaluated or sufficient data were suggested to assess associations. Articles were removed according to the exclusion criteria: (1) non-English or non-Chinese articles; (2) abstracts, conference records, systematic reviews or meta-analysis, and articles without case–control studies; (3) articles with insufficient data to calculate the ORs and 95% CIs; (4) the data originated from the online dataset; (5) articles lacking usable data on genotypes or allele frequencies; and “star”, which was delimited in the 2.3 section judged (6) low-quality articles.
Data extraction and quality assessment
All included articles were identified by two investigators (Jikang Shi and Zhuoshuai Liang). If the two investigators could not agree on an included article, the third investigator (Lingfeng Pan) settled in conformity finally. We collected the following data (first author’s name, publication year, ethnicity, distribution of genotypes and alleles in MI cases and controls, sample sizes of MI cases and controls, and evidence of conforming to the Hardy–Weinberg equilibrium (HWE) among controls). The other information was extracted, such as sex and the last name of the first author. We evaluated the quality of the included articles using the Newcastle–Ottawa scale (NOS). It allocated a score of one point when an included article met a condition; otherwise, no point (0 scores) was allocated. Furthermore, for each included article, the sum of all points (total Quality Score) represented the quality of this article [20]. Low-quality articles were also excluded to avoid selection bias.

Statistical analysis
The association of APOE polymorphisms and myocardial infarction was analyzed using R Studio (Version 1.1.383) (RStudio, Inc., MA, USA). We designated the ε3 allele and ε3/ε3 as the reference and collected the ORs and 95% CIs for evaluating the prognostic value of APOE polymorphisms. The pooled ORs and 95% CIs were estimated in the seven types (ε2/ε2 vs. ε3/ε3, ε2/ε3 vs. ε3/ε3, ε2/ε4 vs. ε3/ε3, ε4ε3 vs. ε3/ε3, ε4/ε4 vs. ε3/ε3, ε2 allele vs. ε3 allele, and ε4 allele vs. ε3 allele).

Hardy–Weinberg equilibrium (HWE) for each included article among control groups was evaluated using the Chi-square test-based Q-statistic and f²-statistic for evaluating heterogeneity. We carried out the comparisons of APOE genotypes, as genotypes can represent the combined effect of alleles. For heterogeneity between studies given by f² > 50%, random-effect models were applied; otherwise, if f² < 50%, fixed-effect models were used [21]. Furthermore, sensitivity analysis was used to assess the stability of articles. The publication bias of this meta-analysis was analyzed using funnel plot and Begg’s test [22].

Trial sequential analysis (TSA)
Traditional meta-analysis is criticized because the data of articles are inevitably clinically diverse among patients, such as ethnicities and diseases states. Systematic bias and random errors result in false-positive results (type I errors) or overestimated treatment effects that may also be obtained by Meta-analyses. Because of neglecting heterogeneity, simply pooling the results is inappropriate [23].

Trial sequential analysis (TSA) provides the required sample size (RIS), analyzing monitoring boundaries of trial sequential if articles do not reach the RIS [24]. The horizontal ordinate is the sample size, and the vertical ordinate is the Z-curve score of the effect. The Z-curve in the upper half of the vertical ordinate indicates a protective effect. Rather, that in the lower half of the vertical ordinate indicates risk effect. The fewer participants and events are, the more restrictive the monitoring boundaries are needed. Furthermore, a much less P-value is required to obtain statistical significance [22]. TSA software (TSA, version 0.9.5.5; Copenhagen Trial Unit, Copenhagen, Denmark, 2016) was used in this Meta-analysis. We set type I error at 5% and type II error at 20% [23]; thus, the statistical power was 80% (power = 1–20%). The relative risk reduction (RRI) was defined as 20%.

Results
Characteristics of studies
We scrutinized 1469 articles according to the inclusion and exclusion criteria, finally selecting 32 articles investigated in this meta-analysis [16, 25–51]. The selected 32 articles provided 13,706 cases with MI and 14,817 controls. (Fig. 1; Table 1).

Quantitative synthesis
In the pooled analysis, the significant heterogeneity between APOE polymorphism and MI risks was found in ε2 vs. ε3 (f² = 65%, P < 0.01) and ε4 vs. ε3 (f² = 76%, P < 0.01). The random-effects model revealed that patients with MI had a low frequency of the ε2 (OR 0.74, 95% CI 0.64–0.86, P < 0.01) (Fig. 2A) and a high frequency of the ε4 (OR 1.24, 95% CI 1.19–1.42, P < 0.01) (Fig. 2B); the pooled OR of ε2/ε3 vs. ε3/ε3 was 0.82 (95% CI 0.76–0.89, P = 0.01) (Fig. 3A); the pooled OR of ε3/ε4 vs. ε3/ε3 was OR 1.20 (95% CI 1.05–1.37, P < 0.01) (Fig. 3B); and the pooled OR of ε4/ε4 vs. ε3/ε3 was OR = 1.31 (95% CI 1.05–1.63, P < 0.01) (Fig. 3C). However, compared with ε3/ε3, ε2/ε2 (Fig. 3D) and ε2/ε4 (Fig. 3E) might not influence MI risks (for ε2/ε2, OR 0.52, 95% CI 0.26–1.01, P < 0.01) (for ε2/ε4, OR 0.96, 95% CI 0.76–1.21, P = 0.48).

Subgroup analysis
To find the potential source of heterogeneity, we ran meta-regression analysis before subgroup analysis, The results show that HWE is a source of heterogeneity
in ε4 vs. ε3 (P = 0.019); in ε3/ε4 vs. ε3/ε3, both HWE (P = 0.0025) and ethnicity (P = 0.0294) are sources of heterogeneity.

We performed subgroup analysis based on the HWE, finding that articles satisfying the HWE had significant heterogeneity. Furthermore, we found that low MI risks existed in carriers of the ε2 allele (OR 0.82, 95% CI 0.74–0.90, P = 0.01) and those of ε2/ε3 vs. ε3/ε3 (OR 0.75, 95% CI 0.67–0.85, P < 0.01); in contrast, high MI risks existed in carriers of the ε4 allele (OR 1.34, 95% CI 1.18–1.52, P < 0.01) and those of ε3/ε4 vs. ε3/ε3 (OR 1.27, 95% CI 1.09–1.48, P < 0.01). In addition, articles not satisfying the HWE had significant heterogeneity (for ε2 allele, P < 0.01; for ε4 allele, P < 0.01; for ε2/ε4 vs. ε3/ε3, P = 0.04; for ε3/ε4 vs. ε3/ε3, P < 0.01; and for ε4/ε4 vs. ε3/ε3, P < 0.01). Moreover, we found that low MI risks existed in carriers of the ε2 allele (OR 0.56, 95% CI 0.40–0.79, P < 0.01), but there were no associations of MI risks with carriers of ε4 allele or with those of ε4-involved (ε2/ε4 vs. ε3/ε3, and ε3/ε4 vs. ε3/ε3) genotypes.

We carried out subgroup analysis based on ethnicity, finding that articles involving Asians had significant heterogeneity. The ε2 allele was a protective factor for MI (P < 0.01, OR 0.70, 95% CI 0.50–0.98); in contrast, the ε4 allele (P < 0.01, OR 1.56, 95% CI 1.04–2.35) and
Table 1  Main characteristics of the included studies

| Study          | Year  | Country | Ethnicity  | Sample size | Quality Score | HWE   | ApoE ε2 (n) | ApoE ε3 (n) | ApoE ε4 (n) |
|----------------|-------|---------|------------|-------------|---------------|-------|-------------|-------------|-------------|
| Cumming et al  | 1984  | Scotland| Scottish   | 239 239     | 7             | Y (P<0.57) | 28 39       | 351 367     | 99 70       |
| Yamamura et al | 1984  | Germany | Caucasian  | 523 1031    | 6             | N (P<0.01) | 93 379      | 826 1594    | 127 90      |
| Utermann et al | 1984  | Japan   | Japanese   | 523 1031    | 5             | N (P<0.01) | 93 379      | 826 1594    | 127 309     |
| Lencz et al    | 1986  | Germany | Caucasian  | 570 624     | 8             | Y (P=0.16) | 63 99       | 907 978     | 170 171     |
| Luc et al      | 1994  | Berlin  | Caucasian  | 183 176     | 7             | Y (P=0.57) | 25 36       | 270 266     | 71 50       |
| Luc et al      | 1994  | Lille   | Caucasian  | 64 150      | 7             | Y (P=0.98) | 6 33        | 105 223     | 17 44       |
| Luc et al      | 1994  | Strasbourg | 187 172    | 7             | Y (P=0.51) | 27 29       | 288 274     | 59 41       |
| Luc et al      | 1994  | Toulouse| Caucasian  | 140 182     | 7             | Y (P=0.84) | 16 20       | 228 311     | 36 33       |
| Joven et al    | 1998  | Spain   | Caucasian  | 250 250     | 6             | Y (P=0.19) | 39 25       | 397 438     | 64 37       |
| Nakai et al    | 1998  | Japan   | Japanese   | 254 422     | 6             | Y (P=0.29) | 12 20       | 418 744     | 66 80       |
| Batalla et al  | 2000  | Spain   | Spanish    | 220 200     | 8             | Y (P=0.89) | 10 19       | 389 348     | 41 33       |
| Zhao et al     | 2000  | Liaoning| Asian      | 50 49       | 7             | Y (P=0.76) | 4 5         | 90 90       | 6 3         |
| Raslová et al  | 2001  | Slovak  | Caucasian  | 71 71       | 6             | Y (P=0.30) | 12 7        | 111 114     | 13 17       |
| Wang et al     | 2001  | Xinjiang| Asian      | 54 106      | 6             | Y (P=0.58) | 3 15        | 82 174      | 23 23       |
| Gong et al     | 2001  | Guangdong| Asian      | 108 115     | 7             | Y (P=0.47) | 14 16       | 170 196     | 52 18       |
| Bai et al      | 2001  | Liaoning| Asian      | 47 113      | 6             | Y (P=0.36) | 4 11        | 90 200      | 6 9         |
| Kolovou et al  | 2002  | Greece  | Greek      | 267 240     | 7             | Y (P=0.72) | 39 39       | 412 392     | 83 49       |
| Mamotte et al  | 2002  | Australia| Caucasian  | 359 639     | 6             | Y (P=1.54) | 39 92       | 554 983     | 125 203     |
| Kumar et al    | 2003  | North India | 35 45     | 5             | Y (P=0.03) | 7 13        | 36 73       | 27 4        |
| Li et al       | 2003  | Nantong | Asian      | 67 152      | 5             | Y (P=0.10) | 16 26       | 98 253      | 22 25       |
| Chen et al     | 2003  | Liaoning| Asian      | 50 110      | 5             | Y (P=0.09) | 4 11        | 90 92       | 6 3         |
| Keaveney et al | 2004  | UK      | Caucasian  | 651 575     | 6             | Y (P<0.01) | 440 686     | 6778 8830   | 1206 1376   |
| Ranjith et al  | 2004  | Indian  | African    | 195 300     | 6             | N (P<0.01) | 10 27       | 330 517     | 50 56       |
| Aaseve et al   | 2006  | Estonia | Caucasian  | 71 85       | 8             | Y (P=0.98) | 7 18        | 110 133     | 23 21       |
| Baum et al     | 2006  | Hongkong| Chinese    | 231 311     | 6             | Y (P=0.81) | 17 70       | 387 505     | 58 47       |
| Koeh et al     | 2008  | Germany | Caucasian  | 3657 1211   | 6             | Y (P=0.72) | 517 201     | 5769 1899   | 1028 322    |
| Vitanen et al  | 2011  | Finland | Caucasian  | 118 110     | 5             | Y (P=0.98) | 7 10        | 171 175     | 58 35       |
| Onsrat et al   | 2012  | Turkey  | Turkish    | 100 36      | 6             | Y (P=0.55) | 12 4        | 172 62      | 16 6        |
| Tunguturi et al| 2012  | India   | Indian     | 202 210     | 8             | Y (P=0.18) | 12 17       | 329 371     | 63 32       |
| Kukavla et al  | 2017  | Russia  | Russians   | 405 198     | 7             | Y (P=0.50) | 68 32       | 698 326     | 44 38       |
| Gupta et al    | 2018  | India   | Indian     | 168 89      | 6             | Y (P=0.54) | 18 4        | 302 165     | 16 9        |
| Hu et al       | 2020  | Jiangxi | Asian      | 53 632      | 7             | N(P<0.02) | 128 28      | 1055 83     | 81 23       |

ε4/ε3 vs. ε3/ε3 (P<0.01, OR 1.44, 95% CI 1.03–2.01) were risk factors for MI. In addition, there were no significant associations of MI risks with carriers of ε2/ε4 vs. ε3/ε3 (P=0.27), with those of ε2/ε2 vs. ε3/ε3 (OR 0.38, 95% CI 0.12–1.20, P=0.16), with those of ε2/ε3 vs. ε3/ε3 (OR 0.85, 95% CI 0.68–1.03, P=0.34), or with those of ε4/ε4 vs. ε3/ε3 (OR 0.91–9.23, P=0.48). Furthermore, we found that articles involving other ethnicities had significant heterogeneity. The ε2 allele was a protective factor for MI (P<0.01, OR 0.78, 95% CI 0.67–0.91); on the contrary, the ε4 allele was a risk factor for MI (P<0.01, OR 1.16, 95% CI 1.04–1.30). There was no significant heterogeneity of MI risks with carriers of ε2/ε4 vs. ε3/ε3 (P=0.09), with those of ε2/ε4 vs. ε3/ε3 (P=0.55), or with those of ε4/ε4 vs. ε3/ε3 (P=0.71). There was no significant association of MI risks with carriers of ε2/ε2 vs. ε3/ε3 (OR 0.59, 95% CI 0.26–1.36, P=0.09) or with those of ε3/ε4 vs. ε3/ε3 (OR 1.13, 95% CI 0.97–1.31, P=0.63).

We carried out subgroup analysis based on the score, finding that articles satisfying the high score had no heterogeneity of MI risks with carriers of the ε2 allele.
(P > 0.05) or with those of ε2-involved genotypes (all P > 0.05). There was no significant association of MI risks with carriers of ε4 vs. ε3 (P < 0.01, OR 1.17, 95% CI 0.90–1.53), with those of ε3/ε4 vs. ε3/ε3 (P < 0.01, OR 1.16, 95% CI 0.91–1.47), or with those of ε4/ε4 vs. ε3/ε3 (P = 0.03, OR 1.32, 95% CI 0.89–1.94). In addition, articles not satisfying the low score showed that all genotypes had significant heterogeneity (all P < 0.01). Low
MI risks existed in carriers of the ε2 allele (P < 0.01, OR 0.70, 95% CI 0.50–0.98); in contrast, high MI risks existed in carriers of the ε4 allele (P < 0.01, OR 1.56, 95% CI 1.04–2.35) or in those of ε3/ε4 vs. ε3/ε3 (P < 0.01, OR 1.22, 95% CI 1.03–1.45). There were no significant associations of MI risks with carriers of ε2/ε2 vs. ε3/ε3 (OR 1.22, 95% CI 1.03–1.40, P > 0.05), with those of ε2/ε3 vs. ε3/ε3 (OR 0.87, 95% CI 0.72–1.60, P > 0.05), or with those of ε4/ε4 vs. ε3/ε3 (OR 1.53, 95% CI 0.91–2.59) (Table 2).

### Sensitivity analysis

To clarify the sources of heterogeneity, sensitivity analyses were performed to assess the stability of the results and the source of the heterogeneity by omitting individual studies and to show the influence of the individual data on the total ORs. Results of sensitivity analysis on the ε2 allele (Fig. 4A), the ε4 allele (Fig. 4B), ε2/ε2 vs. ε3/ε3 (Fig. 4C), ε2/ε3 vs. ε3/ε3 (Fig. 4D), ε2/ε4 vs. ε3/ε3 (Fig. 4E), ε3/ε4 vs. ε3/ε3 (Fig. 4F), and ε4/ε4 vs. ε3/ε3 (Fig. 4G) were presented in Fig. 4. No individual article affected the corresponding pooled ORs and 95% CIs; therefore, the result of this meta-analysis was statistically robust (Tables 3, 4).

### Publication bias

Funnel plots were performed to assess the publication bias and quantified by Begg’s test. The results showed that there was no significant publication bias in neither alleles nor genotypes (all P > 0.05) (Additional file 1: Figure S1).

### Table 2: Subgroup analysis of associations of MI risks with APOE alleles or with genotypes

| Variable | Asian | Other |
|----------|-------|-------|
| Alleles  |       |       |
| ε2       | 0.70  (0.50, 0.98) | 0.78  (0.67, 0.91) |
| ε4       | 1.56  (1.04, 2.35) | 1.16  (1.04, 1.30) |
| Genotypes|       |       |
| ε2/ε2    | 0.38  (0.12, 1.20) | 0.59  (0.26, 1.36) |
| ε2/ε3    | 0.85  (0.60, 1.22) | 0.82  (0.75, 0.90) |
| ε2/ε4    | 0.96  (0.61, 1.51) | 0.96  (0.74, 1.25) |
| ε3/ε4    | 1.44  (1.03, 2.01) | 1.13  (0.97, 1.31) |
| ε4/ε4    | 2.90  (0.91, 9.23) | 1.19  (0.92, 1.55) |

ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε4 and ε4/ε4 were compared with ε3/ε3. ε2 and ε4 were compared with ε3.

Fig. 4 Forest plot of subgroup analysis of the association between APOE alleles/genotypes and myocardial infarction.
Table 3  Sensitivity analysis of associations between APOE alleles and MI risks

| Study            | ε2 (0.68, 0.78) | ε3 (0.68, 0.78) | ε4 (0.68, 0.78) |
|------------------|-----------------|-----------------|-----------------|
| Cumming et al    | 0.73            | 1.13            | 1.14            |
| Yamamura et al   | 0.76            | 1.16            | 1.16            |
| Utermann et al   | 0.76            | 1.16            | 1.16            |
| Lenzen et al     | 0.73            | 1.14            | 1.10            |
| Luc et al        | 0.73            | 1.14            | 1.10            |
| Luc et al        | 0.74            | 1.14            | 1.09            |
| Luc et al        | 0.73            | 1.14            | 1.08            |
| Luc et al        | 0.73            | 1.14            | 1.08            |
| Joven et al      | 0.72            | 1.13            | 1.07            |
| Nakai et al      | 0.73            | 1.13            | 1.08            |
| Batalla et al    | 0.73            | 1.14            | 1.08            |
| Zhao et al       | 0.73            | 1.14            | 1.08            |
| Raslová et al    | 0.73            | 1.14            | 1.08            |
| Wang et al       | 0.73            | 1.14            | 1.08            |
| Gong et al       | 0.73            | 1.14            | 1.08            |
| Bai et al        | 0.73            | 1.14            | 1.08            |
| Kolovou et al    | 0.73            | 1.13            | 1.08            |
| Mamotte et al    | 0.73            | 1.14            | 1.08            |
| Kumar et al      | 0.73            | 1.13            | 1.07            |
| Li et al         | 0.73            | 1.13            | 1.08            |
| Chen et al       | 0.73            | 1.14            | 1.08            |
| Keavney et al    | 0.69            | 1.14            | 1.07            |
| Ranjith et al    | 0.73            | 1.14            | 1.08            |
| Aasvée et al     | 0.73            | 1.14            | 1.08            |
| Baun et al       | 0.74            | 1.13            | 1.08            |
| Koch et al       | 0.71            | 1.16            | 1.09            |
| Vitkanen et al   | 0.73            | 1.13            | 1.08            |
| Onrat et al      | 0.73            | 1.14            | 1.08            |
| Tanguturi et al  | 0.73            | 1.13            | 1.07            |
| Kukava et al     | 0.73            | 1.15            | 1.09            |
| Gupta et al      | 0.73            | 1.14            | 1.08            |
| Hu et al         | 0.74            | 1.15            | 1.09            |

ε2 and ε4 were compared with ε3

Discussion

This meta-analysis, based on up-to-date data, further investigate the association between APOE polymorphism and MI risks, indicating that the ε2 allele and ε2-involved genotypes may be protective factors for MI; in contrast, the ε4 allele and ε4-involved genotypes (ε4/ε3 vs. ε3/ε3, and ε4/ε4 vs. ε3/ε3) may be risk factors for MI.

We found that the genotype ε2/ε2 is associated with MI risks. Of note, Qi et al. observed the genotype ε2/ε2 is not associated with MI risks [53]. Apart from methods that Qi et al. used [53], we adopted TSA additionally. Simple sizes reached RIS, and Z-curves crossed the trial sequential monitoring boundaries, documenting that the association of the genotype ε2/ε2 with MI risks is robust (Fig. 5).

Both the meta-analysis of Luc [29] and our meta-analysis identified that the ε2 allele and ε2-involved genotypes may be implicated in MI as protective factors; in contrast, the ε4 allele and ε4-involved genotypes (ε4/ε3 vs. ε3/ε3, and ε4/ε4 vs. ε3/ε3) may be implicated in MI as risk factors. Luc et al. conducted their meta-analysis based on multicenter population-based case-control study [29]. Population-based articles are more creditable than hospital-based articles and are less frequently performed in other meta-analyses. [18, 29, 40].

Wang et al. observed the genotype ε4/ε4 had no significant association with MI risks [18]. In addition, Kenji et al. and Prabhat et al. both observed the ε2 allele and ε2-involved genotype (ε2/ε2 and ε2/ε3) had no significant association with MI risks [31, 40]. Because we performed TSA, the disagreements may be because the false-negative error was existed in those studies [18, 31, 40]. In addition, Kenji et al. just enrolled Japanese patients [31] and the articles of Prabhat et al. investigated Indian individuals[40]. For these reasons, we performed subgroup analysis stratified by ethnicity, identifying that the association of MI risks with the APOE ε2 allele and with genotypes (ε2/ε2, ε2/ε3) is weaker in Asian than that in other ethnicities. Furthermore, we performed sensitivity analyses and TSA to obtain a reliable conclusion.

TSA

For associations of MI risks with ε2 allele (Additional file 2: Figure S2A), with ε2/ε2 vs. ε3/ε3 (Additional file 2: Figure S2B), and with ε2/ε3 vs. ε3/ε3 (Additional file 2: Figure S2C), simple sizes reached RIS, and Z-curves crossed the trial sequential monitoring boundaries. For associations of MI risks with ε4 allele (Additional file 3: Figure S3A), with ε3/ε4 vs. ε3/ε3 (Additional file 3: Figure S3B), and with ε4/ε4 vs. ε3/ε3 (Additional file 3: Figure S3C), simple sizes reached the RIS but Z-curves did not cross the trial sequential monitoring boundaries.
Our study has some limitations. First, despite subgroup analyses and regression, the main sources of heterogeneity remain difficult to identify. Second, our study focused on articles based on case–control design, merely providing the associations between APOE polymorphism and MI risks, rather than a causal relationship. Third, we did not retrieve other confounding factors, such as the low-density lipoprotein receptor gene, lifestyle, and gene–gene or gene-environment interactions, because the articles included in this meta-analysis did not provide any information about the other confounding factors.

Despite the limitations above, our study has some strengths. First, up-to-date articles were collected extensively, conferring our study more statistical power to draw valid conclusions on the associations between APOE polymorphism and MI risks. Second, the result of sensitivity analysis documented that our conclusions are stable and reliable. Third, in contrast to previous meta-analyses on the association between APOE gene polymorphism and MI risks, this is the first study to use TSA to further build reliable evidence to draw conclusions.

In conclusion, the ε2 allele and ε2-involved genotypes, as protective factors, have been implicated in MI. However, the ε4 allele and ε4-involved genotypes (ε4/ε3 vs. ε3/ε3, and ε4/ε4 vs. ε3/ε3) may perform as risk factors for MI.

### Table 4  Sensitivity analysis of associations between APOE genotypes and MI risks

| Study            | ε2/ε2 | ε2/ε3 | ε2/ε4 | ε3/ε4 | ε4/ε4 |
|------------------|-------|-------|-------|-------|-------|
| Cumming et al    | 0.27  | 0.27  | 0.93  | 1.12  | 1.28  |
| Yamamura et al   | 0.37  | 0.37  | 0.93  | 1.15  | 1.40  |
| Utermann et al   | 0.37  | 0.37  | 0.93  | 1.15  | 1.40  |
| Lenzen et al     | 0.28  | 0.28  | 1.02  | 1.12  | 1.34  |
| Luc et al        | 0.27  | 0.27  | 0.97  | 1.12  | 1.30  |
| Luc et al        | 0.27  | 0.27  | 0.98  | 1.13  | 1.31  |
| Luc et al        | 0.27  | 0.27  | 0.93  | 1.12  | 1.30  |
| Luc et al        | 0.27  | 0.27  | 0.93  | 1.12  | 1.31  |
| Joven et al      | 0.27  | 0.27  | 0.94  | 1.11  | 1.33  |
| Nakai et al      | 0.27  | 0.27  | 0.96  | 1.12  | 1.26  |
| Batallà et al    | 0.27  | 0.27  | 0.96  | 1.13  | 1.30  |
| Zhao et al       | 0.27  | 0.27  | 0.96  | 1.12  | 1.31  |
| Raslová et al    | 0.27  | 0.27  | 0.96  | 1.13  | 1.31  |
| Wang et al       | 0.27  | 0.27  | 0.95  | 1.12  | 1.29  |
| Gom et al        | 0.27  | 0.27  | 0.96  | 1.12  | 1.31  |
| Bai et al        | 0.27  | 0.27  | 0.96  | 1.12  | 1.31  |
| Kolovou et al    | 0.27  | 0.27  | 0.97  | 1.13  | 1.32  |
| Mamotte et al    | 0.25  | 0.25  | 0.97  | 1.12  | 1.34  |
| Kumar et al      | 0.27  | 0.27  | 0.95  | 1.12  | 1.23  |
| Li et al         | 0.26  | 0.26  | 0.95  | 1.12  | 1.29  |
| Chen et al       | 0.27  | 0.27  | 0.96  | 1.12  | 1.31  |
| Keavney et al    | 0.27  | 0.27  | 0.96  | 1.10  | 1.31  |
| Ranjith et al    | 0.27  | 0.27  | 0.95  | 1.12  | 1.34  |
| Aasvee et al     | 0.27  | 0.27  | 0.97  | 1.12  | 1.30  |
| Baum et al       | 0.27  | 0.27  | 0.96  | 1.12  | 1.29  |
| Koch et al       | 0.22  | 0.22  | 0.98  | 1.15  | 1.29  |
| Vitanen et al    | 0.27  | 0.27  | 0.95  | 1.12  | 1.27  |
| Osnat et al      | 0.27  | 0.27  | 0.96  | 1.12  | 1.33  |
| Tanguutti et al  | 0.27  | 0.27  | 0.95  | 1.12  | 1.25  |
| Kukava et al     | 0.26  | 0.26  | 0.97  | 1.14  | 1.30  |
| Gupta et al      | 0.27  | 0.27  | 0.96  | 1.13  | 1.31  |
| Hu et al         | 0.28  | 0.28  | 1.02  | 1.13  | 1.40  |

ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε4 and ε4/ε4 were compared with ε3/ε3
Abbreviations
APOE: Apolipoprotein E; MI: Myocardial infarction; LDL-C: Low-density lipid protein cholesterol; TGs: Triglycerides; SNPs: Single nucleotide polymorphisms; HWE: Hardy–Weinberg equilibrium; NOS: Newcastle–Ottawa scale; CI: Confidence interval; TSA: Trial sequential analysis; RIS: Required sample size.

Supplementary Information
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Additional file 1. Figure S1. Funnel plot of the association between APOE gene polymorphism and myocardial infarction. (A) ε2 allele; (B) ε4 allele; (C) ε2/ε2 genotype; (D) ε2/ε3 genotype; (E) ε2/ε4 genotype; (F) ε3/ε4 genotype; (G) ε4/ε4 genotype.

Additional file 2. Figure S2. Trial sequential analysis of the association between ApoE gene polymorphism and myocardial infarction. (A) ε2 allele; (B) ε2/ε2 genotype; (C) ε2/ε3 genotype.

Additional file 3. Figure S3. Trial sequential analysis of the association between ApoE gene polymorphism and myocardial infarction. (A) ε4 allele; (B) ε3/ε4 genotype; (C) ε4/ε4 genotype.

Additional file 4. Figure S4. Trial sequential analysis of the association between ε2/ε4 genotype and myocardial infarction.

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Authors’ contributions
Conception and design: AYS, YC, and YCQ. Provision of study materials: AYS, JKS, ZSL, and LFP. Collection and assembly of data: AYS, JKS, and ZSL. Data analysis and interpretation: AYS and JKS. Manuscript writing: AYS. Revised the language/article: All authors. Final approval of manuscript: All authors. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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