Association between Apolipoprotein E polymorphism and myocardial infarction risk: A systematic review and meta-analysis

Yi-Lian Wang *,1, Li-Ming Sun 1, Li Zhang, Hai-Tao Xu, Zheng Dong, Luo-Qing Wang, Ming-Lang Wang

Department of Cardiology, The Second People’s Hospital of Lianyungang, Lianyungang 222006, China

ARTICLE INFO

Article history:
Received 1 August 2015
Revised 15 October 2015
Accepted 16 October 2015

Keywords:
Myocardial infarction
MI
Apolipoprotein E
ApoE
Polymorphism
Meta-analysis

ABSTRACT

Published data regarding the association between Apolipoprotein E (ApoE) genetic variation and myocardial infarction (MI) risk were not always consistent. Therefore, the current meta-analysis was conducted to derive a more precise estimation of the association between ApoE polymorphism and MI risk. PubMed and Web of Science were searched to identify relevant studies. Summary odds ratio (ORs) and 95% confidence intervals (CIs) were calculated using random-effect or fixed-effect models based on the heterogeneity of included studies. All the tests were performed using Stata 11.0. A total of 22 eligible studies were identified in this meta-analysis. The results show that ApoE e2 and e4 alleles were associated with MI risk. The study suggests that there is close association between ApoE polymorphism and MI risk. It shows that ApoE e2 allele is a protective factor of MI, while e4 allele is a risk factor of MI, especially in Caucasian and Asian population. Nevertheless, well-designed, unbiased and larger sample size studies are required to confirm the results.

© 2015 The Authors. Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Myocardial infarction (MI) is a complex syndrome affected by multiple predisposing genetic and environmental factors [1]. The association between ApoE polymorphisms and MI has drawn a lot of attention. ApoE is a multifunctional protein which plays a critical role in the metabolism of triglycerides and cholesterol [2,3], and the corresponding gene is considered as a excellent candidate to investigate the etiology of MI [4]. The gene is located at 19q13.2 and possesses three common alleles (e2, e3, e4) and forms six genotypes (e2/e2, e2/e3, e2/e4, e3/e3, e3/e4 and e4/e4) [5]. As the previous studies, the ApoE polymorphisms were found to affect ApoE transcription and the levels of cholesterol and triglyceride [6,7], which was the main underlying risk factor of MI. However, the results of the earlier studies were inconsistent. Therefore, a systematic review and meta-analysis by collecting and sorting the previously published studies was conducted.

2. Materials and methods

2.1. Identification and eligibility of relevant studies

The online medical databases PubMed and Web of Science were used, using the search term “ApoE/Apolipoprotein E”, “polymorphism/genetic variation” and “myocardial infarction/MI”. The last retrieval was conducted in January 2015. The literatures were limited to papers in English. In addition, studies were identified by manual search of the references listed in the retrieved studies. The inclusion criteria were listed as follows: (1) case–control studies with either a population-based or a hospital-based design; (2) studies evaluated association between the ApoE polymorphisms and cancer risk; (3) present sufficient data to calculate an odds ratio (OR) with 95% confidence interval (CI); (4) not republished data. Moreover, the studies without raw data or those that were case-only studies, case reports, editorials and review articles (including meta-analyses) were eliminated.

2.2. Data extraction

The following detail information were extracted from each study enrolled in this study by two investigators (YLW and LZ) independently: the first author’s last name, year of publication, country of subjects, ethnicity, the source of controls, genotyping method, matching numbers of genotyped cases and controls and

Abbreviations: ApoE, Apolipoprotein E; MI, myocardial infarction; OR, odds ratio; CIs, confidence intervals; HWE, Hardy–Weinberg equilibrium

* Corresponding author. Tel./fax: +86 0512522345208.
E-mail address: fangxc21@163.com (Y.-L. Wang).
1 These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.fob.2015.10.006
2211-5463 © 2015 The Authors. Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
for Hardy–Weinberg equilibrium (HWE). Furthermore, the disagreements were discussed among all authors and resolved with consensus.

2.3. Statistical analysis

The association of the ApoE polymorphism and risk of myocardial infarction was estimated by calculating the pooled ORs and 95%CI. The pooled ORs were estimated for seven genetic models (e2/e2 vs. e3/e3, e2/e3 vs. e3/e3, e2/e4 vs. e3/e3, e3/e3 vs. e3/e3, e4/e4 vs. e3/e3, e2 allele vs. e3 allele and e4 allele vs. e3 allele).

Stratified analyses were performed by ethnicity (‘other ethnicity’ group was defined as those ethnicities that contained only one study). Heterogeneity across the studies was evaluated by using the Chi-square test based Q-statistic test [8], and it was considered significant when \( P_{\text{heterogeneity}} < 0.05 \). The data were combined using random-effects (the DerSimonian and Laird method) in the presence of heterogeneity (\( P < 0.05 \) or \( I^2 > 50\% \)) and fixed-effects (the Mantel–Haenszel method) models were used in absence of heterogeneity (\( P > 0.05 \) or \( I^2 < 50\% \)) [9]. Furthermore, the sensitivity analysis was used to assess the stability of results, and publication bias was analyzed by Begg’s funnel plot and Egger’s regression test.

![Flow diagram of the study selection process.](image)

**Table 1**

The characteristics of the enrolled studies in this meta-analysis.

| First author | Year | Country | Ethnicity | Source of control | Genotyping method | Sample size (cases/controls) | HWE   |
|--------------|------|---------|-----------|-------------------|-------------------|-----------------------------|-------|
| Tanguturi    | 2013 | India   | Asian     | PB                | PCR-RFLP          | 202/210                     | 0.097 |
| Anand        | 2009 | Mixed   | Mixed     | Mixed             | IlluminaGoldenGate technology | 4017/4017                  | 0.091 |
| Al-Bustan    | 2009 | Kuwaiti | Asian     | HB                | PCR-RFLP          | 88/122                      | <0.050|
| Koch         | 2008 | Germany | Caucasian | PB                | TaqMan            | 3657/1211                  | 0.558 |
| Ranjith      | 2004 | Indian  | African   | PB                | PCR-RFLP          | 195/300                    | <0.050|
| Keavney      | 2004 | UK      | Caucasian | PB                | PCR-RFLP          | 4685/3460                  | –     |
| Keavney      | 2003 | UK      | Caucasian | PB                | PCR-RFLP          | 4484/5757                  | 0.463 |
| Mamotte      | 2003 | Australia | Caucasian | PB                | PCR-RFLP          | 359/639                    | 0.732 |
| Wang         | 2001 | Xinjiang| Asian     | PB                | PCR-RFLP          | 54/71                      | 0.479 |
| Raslova      | 2001 | Bratislava | Caucasian | PB                | PCR-RFLP          | 71/71                      | 0.183 |
| Batalla      | 2000 | Asturias | Caucasian | PB                | PCR-RFLP          | 220/200                    | 0.776 |
| Joven        | 1998 | Spanish | Caucasian | PB                | PCR-RFLP          | 250/250                    | 0.109 |
| Luc          | 1994 | Belfast | Caucasian | PB                | NA                | 183/176                    | 0.405 |
| Luc          | 1994 | Lille   | Caucasian | PB                | NA                | 64/150                     | 0.932 |
| Luc          | 1994 | Strasbourg | Caucasian | PB                | NA                | 187/172                    | 0.35  |
| Luc          | 1994 | Toulouse | Caucasian | PB                | NA                | 140/182                    | 0.698 |
| Lenzen       | 1986 | NA      | Caucasian | PB                | NA                | 570/624                    | 0.081 |
| Kolovou      | 2002 | Greek   | Caucasian | PB                | PCR-RFLP          | 124/240                    | 0.552 |
| Kumar        | 2003 | North India | Asian   | PB                | PCR-RFLP          | 35/45                      | <0.050|
| Baum         | 2006 | Hong Kong | Asian     | PB                | PCR-RFLP          | 234/336                    | 0.659 |
| Nakai        | 1998 | Japan   | Asian     | PB                | PCR-RFLP          | 254/422                    | 0.175 |
| Hergenc      | 1995 | Turkish | Caucasian | PB                | PCR-RFLP          | 50/60                      | 0.117 |
| Utermann     | 1984 | Germany | Caucasian | PB                | NA                | 523/1031                   | <0.050|

NA: not available; PB: population based; HB: hospital based; PCR-RFLP: restriction fragment length polymorphism; HWE: Hardy–Weinberg equilibrium. -: the data of the study are not enough.
Additionally, HWE was used to assess the genotype frequencies of the polymorphism by the chi-square test. All statistical tests were performed with STATA 11.0 and all the P values were two-sided.

3. Results

3.1. Characteristics of studies

Based on the search strategy, 571 potentially eligible studies were identified in the initial search. Among these, 22 studies were enrolled in this meta-analysis based on the inclusion criteria [11–32] (Fig. 1). The study by Luc et al. [30] investigated in four countries and was divided into four studies. The main characteristics of the enrolled 22 studies are summarized in Table 1.

3.2. Quantitative synthesis

In the pooled analysis, significant association was observed between ApoE polymorphism and risk of myocardial infarction. The main results were presented in Table 2, and the result of e2/e3 vs. e3/e3 was also shown in Fig. 2.

Additionally, the subgroup analysis by ethnicity was also conducted, and significant associations with myocardial infarction were observed in Caucasian (Fig. 3) and Asian population. The main results were presented in Table 2.

3.3. Sensitivity analysis

To assess the stability of the results and assess the source of the heterogeneity, the sensitivity analysis was performed by omitting individual eligible study to reflect the influence of the individual data on the summary ORs. The pooled ORs were not altered for all comparison models. Among all the enrolled studies, four studies did not follow HWE, the corresponding summary ORs were not materially altered with or without these studies. Therefore, the results of current study were statistically robust. The result of e2/e2 vs. e3/e3 was shown in Fig. 4.

3.4. Heterogeneity analysis

There was significant between-study heterogeneity in e2/e2 vs. e3/e3 (P = 0.001, I² = 63.4), e2/e3 vs. e3/e3 (P = 0.005, I² = 48.3), e2/e4 vs. e3/e3 (P = 0.000, I² = 58.0), e2 allele vs. e3 allele (P = 0.000, I² = 64.3) and e4 allele vs. e3 allele (P = 0.000, I² = 65.5). In contrast, no significant heterogeneity was observed in other two genetic models (e2/e4 vs. e3/e3: P = 0.750, I² = 0.0; e4/e4 vs. e3/e3: P = 0.194, I² = 20.6). In order to detect the sources of heterogeneity, the sensitivity analysis was performed based on HWE and ethnicity. However, the heterogeneity was not materially altered. As a consequence, we conducted a Galbraith plot to graphically assess the source of heterogeneity. The results indicated that a total of eight studies contributed to the heterogeneity. Two studies were the main sources for e2/e2 vs. e3/e3 [20,32] (Fig. 5), three studies for e2/e3 vs. e3/e3 [15,21,27], four studies for e3/e4 vs. e3/e3 [16,19,27,32], four studies for e2 allele vs. e3 allele [15,21,27,32] and five studies for e4 allele vs. e3 allele [11,16,19,27,32], and after removal of these outlier studies, the heterogeneity was effectively removed (e2/e2 vs. e3/e3: P = 0.640, I² = 0.0; e2/e3 vs. e3/e3: P = 0.828, I² = 0.0; e3/e4 vs. e3/e3: P = 0.800, I² = 0.0; e2 allele vs. e3 allele: P = 0.651, I² = 0.0; e4 allele vs. e3 allele: P = 0.566, I² = 0.0). Meanwhile, the corresponding pooled ORs were not materially altered in all comparisons. As a consequence, the results of heterogeneity analysis indicated that our results were statistically robust and credible.
3.5. Publication bias

To evaluate the publication bias of enrolled studies, the Beggs’s funnel plot and Egger’s test were performed. The shapes of funnel plots did not show any obvious asymmetry in all genetic models. Therefore, the Egger’s test was performed to provide statistical evidence of funnel plot symmetry, and the results confirmed the absence of publication bias (Table 3).

![Fig. 2. Forest plot for ApoE polymorphism and MI risk in the genetic model of e2/e3 vs. e3/e3.](image)

![Fig. 3. Forest plot for ApoE polymorphism and MI risk among the Caucasian population in the genetic model of e2/e3 vs. e3/e3.](image)
4. Discussion

A total of 22 studies were included in this meta-analysis to investigate the association between ApoE polymorphisms and MI. The results of the overall studies showed that the $\varepsilon2/\varepsilon2$ genotype was associated with a decreased risk of MI, while the $\varepsilon3/\varepsilon4$ and $\varepsilon4/\varepsilon4$ genotypes were associated with an increased risk of MI. The current results were in accord with the previous observers, which support the $\varepsilon4$ allele as a risk factor of MI \cite{16,33,34}. For the $\varepsilon2$ allele, it was found significantly protective against MI \cite{12,21}. The discrepancy of the effect on MI risk between different ApoE genotypes might be supported by earlier studies \cite{35,36}. It provided evidence that the ApoE E4 coding by $\varepsilon4$ allele exhibits enhanced transfer from HDL to TG-rich lipoproteins, promoting hepatic remnant clearance by apoE receptors and decreasing LDLR, thereby increasing cholesterol levels. On the contrary, the ApoE E2 coding by $\varepsilon2$ allele binds LDLR poorly, which can increase the LDLR numbers, thereby lowering cholesterol level. A previous meta-analysis showed no significant association between $\varepsilon2$ carriers and MI risk ($\varepsilon2$ carriers vs. $\varepsilon3/\varepsilon3$: OR = 0.90, 95%CI = 0.76–1.06, $P$ = 0.120), whereas an increased MI risk with $\varepsilon4$ carriers ($\varepsilon4$ carriers vs. $\varepsilon3/\varepsilon3$: OR = 1.18, 95%CI = 1.05–1.33, $P$ = 0.003) \cite{37}. By comparison, our results were not completely consistent with previous meta-analysis. The discrepancy may partly result from the genetic diversity among ethnicities.

Table 3

| Egger's test | $\varepsilon2/\varepsilon2$ vs. $\varepsilon3/\varepsilon3$ | $\varepsilon2/\varepsilon3$ vs. $\varepsilon3/\varepsilon3$ | $\varepsilon2/\varepsilon4$ vs. $\varepsilon3/\varepsilon3$ | $\varepsilon3/\varepsilon4$ vs. $\varepsilon3/\varepsilon3$ | $\varepsilon4/\varepsilon4$ vs. $\varepsilon3/\varepsilon3$ |
|--------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| $t$          | -0.53                           | -0.73                           | 0.6                             | 1.46                            | 0.48                            |
| $p$          | 0.607                           | 0.474                           | 0.554                           | 0.159                           | 0.638                           |

Fig. 4. The sensitivity analysis in the genetic model of $\varepsilon2/\varepsilon2$ vs. $\varepsilon3/\varepsilon3$. The omitted study is indicated by the first author's last name.

Fig. 5. Galbraith plot for ApoE gene polymorphism and MI risk in the genetic model of $\varepsilon2/\varepsilon2$ vs. $\varepsilon3/\varepsilon3$. 

Egger’s test for ApoE polymorphism.
Furthermore, the subgroup analysis by ethnicity showed a decreased MI risk in ε2 carriers, while an increased MI risk in ε4 carriers compared with ε3 carriers in both Asian and Caucasian population. These results were consistent with the studies enrolled in our meta-analysis [11,12,15,19,26,30]. Furthermore, sensitivity analysis was also performed to make sure whether modification of the inclusion criteria of the meta-analysis affected the final results. The results showed that corresponding pooled ORs were not materially altered in all genetic models which indicated that the results were statistically robust.

Heterogeneity is a potentially important factor to influence the interpretation of the current results. In this meta-analysis, significant heterogeneity existed in ε2/ε2 vs. ε3/ε3, ε2/ε3 vs. ε3/ε3, ε3/ε4 vs. ε3/ε3, ε2 allele vs. ε3 allele and ε4 allele vs. ε3 allele. Common reasons of heterogeneity may attribute to the diversity in design, study quality, sample-sizes, genotyping methods, inclusion criteria and some studies without HWE. To explore the sources of heterogeneity, we first performed the sensitivity analyses based on HWE and ethnicity. However, the heterogeneity was not effectively removed. Therefore, a Galbraith plot was performed to further evaluate the source of heterogeneity. After excluding eight outlier studies, the heterogeneity was effectively removed. Moreover, the corresponding pooled ORs were not materially altered in all comparisons, which also suggested that our results were statistically robust.

Some limitations of the meta-analysis should be addressed. Firstly, the potential factors such as gender, age, smoking, drinking, living habits were not considered in this meta-analysis. Secondly, between-study heterogeneity should be paid attention, which may affect the results. Thirdly, only studies in English were enrolled in this meta-analysis, which may lose some studies in other languages consistent with inclusion criteria. Regardless of such limitations, this meta-analysis still had some advantages. Firstly, all enrolled studies were consistent with inclusion criteria well. Secondly, no publication bias was observed indicating that the whole pooled results might be unbiased.

In conclusion, the current meta-analysis of 22 studies indicated that ApoE ε2 allele was a protective factor of MI, while ε4 allele was a dangerous factor of MI, especially in Asian and Caucasian population. However, the results should be further confirmed in well-designed, unbiased, powered studies.

Author contributions
YLW conceived and designed the project. LMS, LZ and HTX acquired the data. ZD and QZ analyzed and interpreted the data. YLW and MLW wrote the paper.

Conflict of interest
The authors declare that they have no competing interests.

Acknowledgment
None.

References
[1] Luo, J.Q., Wen, J.G., Zhou, H.H., Chen, X.P. and Zhang, W. (2014) Endothelial nitric oxide synthase gene g984t polymorphism and myocardial infarction: a meta-analysis of 34 studies involving 21068 subjects. PLoS One 9 (1); e87196.
[2] Mozaffar, P., Castillo, S., Reyes, C., et al. (2003) Apolipoprotein E polymorphism in Brazilian dyslipidemic individuals: oto preto study. Braz. J. Med. Biol. Res. 40 (1); 49-56.
[3] Stampfer, M.J., Sacks, F.M., Salovini, S., Willett, W.C. and Hennekens, C.H. (1991) A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. N. Engl. J. Med. 325 (6); 373-381.
[4] Lahiri, D.K., Sambamurti, K. and Bennett, D.A. (2004) Apolipoprotein gene and its interaction with the environmentally driven risk factors: molecular, genetic and epidemiological studies of Alzheimer's disease. Neurobiol. Aging 25 (5); 655-660.
[5] Pollin, T.I., Huseh, W.C., Steinle, N.I., Snitzer, S., Shuldiner, A.R. and Mitchell, B. D. (2004) A genome-wide scan of serum lipid levels in the old order amish. Atherosclerosis 173 (1); 89-96.
[6] Gilt, G., Chen, W.M., Scherer, S.E. et al. (2006) Heritability of cardiovascular and personality traits in 6,148 Sardinians. PLoS Genet. 2 (8); e132.
[7] Handoll, H.H. (2006) Systematic reviews on rehabilitation interventions. Arch. Phys. Med. Rehabil. 87 (6), 875.
[8] Mijdert, A.S., Woude, K.C., Strijbosch, J.R., Porath, A., Fleming, C. and Pauker, S. G. (1994) Cost-effectiveness of streptokinase for acute myocardial infarction: a combined meta-analysis and decision analysis of the effects of infarct location and of likelihood of infarction. Med. Decis. Making 14 (2); 108-117.
[9] Egger, M., Davey Smith, G., Schneider, M. and Minder, C. (1997) Bias in meta-analysis detected in 23% of the results. Lancet 349 (9055); 1029-1032.
[10] Yuan, Y.L., Wang et al. / FEBS Open Bio 5 (2015) 852–858
[31] Lenzen, H.J., Assmann, G., Buchwalsky, R. and Schulte, H. (1986) Association of apolipoprotein E polymorphism, low-density lipoprotein cholesterol, and coronary artery disease. Clin. Chem. 32 (5), 778–781.

[32] Utermann, G., Hardewig, A. and Zimmer, F. (1984) Apolipoprotein E phenotypes in patients with myocardial infarction. Hum. Genet. 65 (3), 237–241.

[33] Lehtinen, S., Lehtimaki, T., Sisto, T., et al. (1995) Apolipoprotein E polymorphism, serum lipids, myocardial infarction and severity of angiographically verified coronary artery disease in men and women. Atherosclerosis 114 (1), 83–91.

[34] Singh, P.P., Singh, M., Bhatnagar, D.P., Kaur, T.P. and Gaur, S.K. (2008) Apolipoprotein E polymorphism and its relation to plasma lipids in coronary heart disease. Indian J. Med. Sci. 62 (3), 105–112.

[35] Davignon, J., Gregg, R.E. and Sing, C.F. (1988) Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 8 (1), 1–21.

[36] Lahoz, C., Schaefer, E.J., Cupples, L.A., et al. (2001) Apolipoprotein E genotype and cardiovascular disease in the Framingham Heart Study. Atherosclerosis 154 (3), 529–537.

[37] Song, Y., Stampfer, M.J. and Liu, S. (2004) Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann. Intern. Med. 141 (2), 137–147.