# Asian Journal of Epidemiology

ISSN 1992-1462

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OPEN ACCESS Asian Journal of Epidemiology

ISSN 1992-1462
DOI: 10.3923/aje.2017.54.62

# Research Article
# Relation Between apoE Gene Polymorphism and Coronary Artery Disease in South East and East Asian Countries

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## Abstract

**Background and Objective:** Apolipoprotein E (apoE) is a polymorphic protein with vital antioxidant and anti-atherosclerotic effects. Three apoE isoforms exist due to polymorphisms in its gene causing disturbances of lipoproteins metabolism and probability to develop cardiovascular diseases. The aim of this study was to assess the association between apoE gene polymorphism and Coronary Artery Disease (CAD) in a Malaysian population sample. Also, to integrate the study findings with other studies to increase the power of the study sample and to make a better understanding about the association between apoE gene polymorphism and CAD in Southeast and East Asian countries. **Methodology:** The study involved 185 patients with CAD attending HTAA hospital Kuantan, Pahang with 188 unrelated healthy control participants. The apoE gene polymorphism was determined in the participants using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay and was validated using direct nucleotide sequencing. The SPSS software version 19 and Chi-squared test was used for determination of allele and genotypes association with CAD. Binary logistic regression analysis of apoE genotypes, gender, ethnicity, age, blood pressure and lipid profile was used to predict the probability of developing CAD. Also, a meta-analysis was conducted using Review Manager (Version 5.3.). **Results:** The preliminary data has shown a non-significant association between apoE genotypes or alleles and CAD. Nevertheless, binary logistic regression analysis has shown that *E3E4* genotype, high blood pressure, male gender and old age are dependent risk factors that significantly predict the occurrence of CAD in the population sample ($p<0.01$). The meta-analysis of studies in Southeast Asia and East Asia region had shown that carriers of the *E4* allele are significantly at higher risk to develop CAD [$p<0.0001$, OR = 1.51 (1.24, 1.83) CI = 95%, $I^2 = 68\%$]. **Conclusion:** This study provides an evidence of increased risk to develop CAD among carriers of *E4* allele especially if accompanied by high blood pressure, old age with the male gender.

Key words: Coronary artery disease, lipoprotein, apolipoprotein E, polymorphism, meta-analysis

Received: January 19, 2017 Accepted: March 06, 2017 Published: March 15, 2017

Citation: Wisam Nabeel Ibrahim, Farrah Wahida Aladzemi, Nurul Ashikin Muhammad Musa, Nik Nur Fatnoon Nik Ahmad, Nor Zamzila Binti Abdullah and Norlelawati Binti Talib, 2017. Relation between apoE gene polymorphism and coronary artery disease in South East and East Asian countries. Asian J. Epidemiol., 10: 54-62.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Coronary Artery Disease (CAD) is well known for its high morbidity and mortality rates that keep rising due to the changes in lifestyle, food habit with other environmental changes among populations in the developed and developing countries[1]. According to World health Organization report published in 2014, CAD counted the highest among the cause-specific death rates in Malaysia reaching approximately 23.10%[2]. The known risk factors for this disease include hypertension, diabetes, smoking, family history, obesity, elevated LDL cholesterol and sedentary life style[1].

Many family and epidemiological studies have shown that genetic predisposition to CAD might be accountable for up to 60% in CAD patients[3]. The genetic predisposition is high due to the heritability of many risk factors such as diabetes[4], hypertension[5], obesity[6] and disturbances of lipoproteins metabolism that explains why CAD cases aggregate among family members[7]. However, there is growing evidence through many genome-wide association studies which had investigated hundreds genomic loci of polymorphisms and confirmed their role in precipitating CAD[8].

The apoE is a polymorphic lipoprotein with crucial biological functions in the protection of the cardiovascular system[9]. However, the role of apolipoprotein E is highly regulated by polymorphisms in its gene[10]. The apoE glycoprotein acts as a ligand for LDL receptor regulating the transport of cholesterol and other lipids among various cells of the body[10]. Also, it presents in VLDL, HDL, chylomicrons and Intermediate-Density Lipoprotein (IDLs) to regulate triglyceride-rich lipoprotein catabolism[10]. The apoE is also known to have powerful antioxidant and anti-inflammatory effects with many anti-atherogenic effects including inhibition of platelets aggregation, prevention of lipid peroxidation and inhibition of vascular smooth muscle proliferation[10].

The significant genetic polymorphisms affecting apoE gene in chromosome 19 involve two substitution polymorphisms in codon 112 and codon 158 giving rise to three alleles, epsilons or isoforms: *E2*, *E3* and *E4* [10]. In each of these epsilons, the codons amino acid products are cysteine-cysteine, cysteine-arginine and arginine-arginine respectively[10]. These three alleles generate six different genotypes with specific disease predisposition[11] whereby, the *E2E2* genotypes is associated with type III hyperlipoproteinemia[10] and the *E4E4* genotype is associated with coronary artery disease and Alzheimer's disease[10,11].

The association of apoE with CAD had been addressed in different populations but results from these populations are not consistent. In addition, the Malaysian figure of apoE genotypes distribution and its role as a risk factor for CAD is not confirmed. Therefore, the aim of this study was to assess the distribution of apoE genotypes, lipid profiles and other risk factors among CAD patients in a Malaysian population sample following a case- control study design. Also, the study attempted to identify any significant interaction among the different risk factors in the prediction of CAD by using binomial logistic regression analysis. Additionally, the study involved meta- analysis to increase the sampling power and to project the study findings within a broader sample by analyzing studies done in East Asian and Southeast Asian populations.

## MATERIALS AND METHODS

Recruitment of the CAD patients took place at the Tengku Ampuan Afzan Hospital, in Kuantan, Pahang (HTAA) between 2011 and 2015. All the molecular laboratory procedures took place in the molecular laboratory in the faculty of medicine, International Islamic University between January and July 2015.

**Study population:** The study started after receiving the ethical approval by the Medical Research and Ethics Committee, Ministry of Health, Malaysia with the registration number of (NMMR 10-495-5071), in addition to the approval by IIUM Research Ethical Committee (IREC). The sample size was calculated using the OpenEpi software adapting the results from Wei *et al.*[12] for unmatched case-control study. The study population comprised 185 unrelated CAD patients (152 males and 33 females) and 188 healthy controls (122 males and 66 females). The diagnosis of CAD was confirmed in the patients for at least three months by the physician before being selected. The healthy controls were unrelated volunteers with no history of CAD or chest pain. After explaining all the necessary details about the study, the participants or their guardians were voluntarily asked to sign the consent forms before filling up the questionnaire. The questionnaire form was used to record information such as age, gender and ethnicity. Medical histories such as the history of hypertension, diabetes, coronary artery disease, smoking and lifestyle were also recorded.

**Biochemical tests:** The lipid profile parameters included the level of Total Cholesterol (TC), High-Density Lipoprotein (HDL) cholesterol, Low-Density Lipoprotein (LDL) cholesterol and triglyceride (TG). The TC, HDL and TG analysis were performed

using Cobas Integra 400 Plus System (Roche). The serum LDL level was calculated using Friedewald formula[13]:

$$\text{LDL (mg dL}^{-1}) = \text{TC (mg dL}^{-1})\text{-HDL (mg dL}^{-1})\text{-[TG (mg dL}^{-1})/5] \quad (1)$$

**Genotyping:** After the collection of the patients' data, 5 mL of the drawn blood was kept into ethylenediamine acetic acid (EDTA) tube. The blood was then centrifuged to get the buffy coat and stored at -20°C until used. The DNA extraction and purification were done by using QIAmp Blood purification kit (Qiagen, Germany) following the manufacturer's protocol. The DNA was also tested for its quantity and quality using bio-photometer plus (Eppendorf, USA).

The apoE genotyping was determined using PCR-RFLP assay. The target sequence of apoE gene was amplified in 15 µL reaction volume that included; 0.25 µM of each primer, 200 µM of dNTPs, I U of One Taq™ Polymerase (New England Biolabs), 1X reaction buffer, 3 mM of $MgCl_2$ and 30 ng of DNA. The primers and the thermal setting of the PCR were similar to the condition used in the study conducted by Ibrahim *et al.*[14]. Upon successful amplification that was confirmed by agarose gel electrophoresis, the PCR products were then digested overnight using HhaI restriction enzyme at 37°C; then the results were analyzed on a 5% agarose gel stained with ethidium bromide and visualized using the gel documentation system (Bio-Rad, USA). The different apoE genotypes were determined and were further validated by sequencing as been mentioned in the previous study[14].

**Statistical analysis:** Microsoft office Excel 2007 (12.0.6766.5000) was used to generate preliminary tables. The statistical analysis was completed using IBM SPSS software version 19 in which apoE genotype and allele frequency distribution was cross-tabulated between the patients and control groups using Chi-square (X2) test. Hardy-Weinberg equilibrium was validated for apoE genotype distribution in the control group by the Chi-square (X2) test with 1 df (p-value = 0.11). The association between apolipoprotein E genotypes and different lipoproteins was tested by one-way ANOVA with Tukey *post hoc* analysis[15]. Numerical data were expressed as the Mean±Standard Deviation (SD) and a p-value of <0.05 was considered statistically significant.

To accurately evaluate the interactions among the different risk factors to predict development of CAD, binary logistic regression analysis was conducted on some predictors using IBM SPSS software version 19.

**Meta-analysis:** To overcome the consequences of small sample selection and for a better understanding about apoE polymorphism in CAD, meta- analysis was conducted. The search process involved scriutinizing online databases including PubMed, Google Scholar, Web of Science and Scopus. The keywords used in these online databases included apolipoprotein E or apoE gene polymorphism and myocardial infarction, MI, coronary artery disease, CAD, ischemic heart diseases or IHD. After screening, a total of 18 studies including our study were selected for the meta-analysis[12,16-31].

The criteria for selection included published and unpublished case-control studies conducted in Southeast or East Asian countries. This approach was supported by the evidence of genetic similarity among certain Asian populations as been claimed in some studies[32,33]. Similar case-control studies conducted in other regions were excluded from the analysis in addition to any study having incomplete or missing data. All the selected studies included determination of apoE gene polymorphism among CAD patients and control participants. By combining results from the original study with the selected studies from China, Japan, Korea, Hong Kong, Thailand, Taiwan and Malaysia, the sample size had more than 95% sampling power to detect a genetic risk factor of OR = 1.51 ($\alpha = 0.05$) based on the finding of Wei *et al.*[12]. In each study, the Odds Ratios (ORs) have been calculated. Then the pooled odds ratios were analyzed using Review Manager Version 5.3. The details of the studies are listed in Table 1.

## RESULTS

**Demographic analysis:** As shown in Table 2, there was a significant difference between the mean ages of CAD patients and control participants (p-value = 0.001). Additionally, there was a significant gender distribution difference between the two groups (p-value = 0.001). The ethnic composition of the participants was mainly of Malays (82%), followed by Chinese (12%) and Indians (6%). The ethnic distributions were significantly different between the two groups (p = 0.02). The ethnic allocation of the participants in the patients group comprised of 74.2, 16.6 and 9.2% of Malays, Chinese and Indians, respectively while the control group had 88.4, 7.4 and 4.2%, respectively.

**Biochemical tests:** The biochemical findings of the study participants are shown in Table 2. The CAD patients had a

Table 1: East Asian and Southeast Asian studies sample sizes and distribution of apoE alleles with their respective Hardy-Weinberg test results

| | | | CAD | | | Control | | | |
|---|---|---|---|---|---|---|---|---|---|
| Studies | CAD | Control | *E2* | *E3* | *E4* | *E2* | *E3* | *E4* | Hardy-Weinberg test in controls |
| Baum *et al.*[16] | 462 | 622 | 17 | 387 | 58 | 70 | 505 | 47 | 0.113 |
| Chaudhary *et al.*[17] | 294 | 298 | 12 | 232 | 47 | 15 | 259 | 21 | 0.051 |
| Eto *et al.*[18] | 218 | 1152 | 18 | 163 | 37 | 43 | 975 | 135 | 0.037* |
| Kim *et al.*[19] | 456 | 274 | 39 | 376 | 41 | 31 | 227 | 16 | 0.113 |
| Kim *et al.*[20] | 242 | 264 | 22 | 186 | 22 | 18 | 201 | 41 | 0.069 |
| Nakai *et al.*[21] | 508 | 440 | 12 | 424 | 72 | 11 | 388 | 41 | 0.026* |
| Nakata *et al.*[22] | 140 | 240 | 7 | 117 | 16 | 18 | 210 | 12 | 0.075 |
| Ou *et al.*[23] | 466 | 704 | 23 | 382 | 56 | 28 | 634 | 42 | 0.041* |
| Ouyang *et al.*[24] | 200 | 100 | 31 | 291 | 78 | 18 | 163 | 19 | 0.090 |
| Peng *et al.*[25] | 426 | 360 | 34 | 328 | 64 | 30 | 303 | 27 | 0.084 |
| Peng *et al.*[26] | 300 | 314 | 24 | 237 | 39 | 16 | 275 | 23 | 0.051 |
| Kim *et al.*[27] | 272 | 714 | 24 | 209 | 38 | 64 | 571 | 79 | 0.090 |
| Wang *et al.*[28] | 186 | 350 | 27 | 291 | 54 | 50 | 601 | 49 | 0.072 |
| Wei *et al.*[12] | 594 | 594 | 107 | 396 | 91 | 47 | 427 | 120 | 0.080 |
| Wu *et al.*[29] | 344 | 572 | 17 | 303 | 24 | 43 | 484 | 45 | 0.080 |
| Yamamura *et al.*[30] | 312 | 416 | 17 | 256 | 39 | 28 | 345 | 43 | 0.068 |
| Yang *et al.*[31] | 408 | 272 | 20 | 348 | 40 | 14 | 239 | 17 | 0.052 |

*E2*: Carriers of apoE epsilon 2 allele, *E3*: Carriers of apoE epsilon 3 allele, *E4*: Carriers of apoE epsilon 4 allele, *Significant difference, $p<0.05$ is statically significant at 95% confident interval, CAD: Coronary artery disease

Table 2: Demographic, clinical and biochemical characteristics of the study participants

| Characteristics | CAD patients | Controls | p-values |
|---|---|---|---|
| Age (years) | 60.00±10.5 | 48.00±5.70 | 0.000* |
| BMI (kg $m^{-2}$) | 26.80±4.50 | 26.60±4.60 | 0.578 |
| Systolic Bp (mmHg) | 131.00±18.5 | 121.00±8.80 | 0.000* |
| Diastolic Bp (mmHg) | 77.00±9.60 | 77.00±800 | 0.200 |
| TC (mmole $L^{-1}$) | 5.20±1.50 | 6.07±1.20 | 0.016* |
| TG (mmole $L^{-1}$) | 2.00±1.10 | 1.40±0.80 | 0.006* |
| LDL (mmole $L^{-1}$) | 3.30±1.30 | 4.05±1.12 | 0.118 |
| HDL (mmole $L^{-1}$) | 0.94±0.37 | 1.34±0.34 | 0.400 |

Mean values±SEM, *Significant difference, $p<0.05$ is taken as statically significant at 95% confident interval, BMI: Body mass index, TC: Total cholesterol, TG: Triglyceride, LDL: Low density lipoprotein, HDL; High density lipoprotein, CAD: Coronary artery disease

significantly high level of serum triglyceride and low level of HDL cholesterol in comparison with control participants (p-value = 0.006). While, the control participants had significantly high TC level than what the CAD patients had (p-value = 0.016). The details of the analysis are shown in Table 2. There was a significant association between ethnicity and the lipid profile in which Malays had significantly higher TC and LDL levels than the Chinese with a p-value of 0.022, 0.04, respectively. Also, the BMI mean values were significantly higher in Malays than in the Chinese (p-value = 0.03, BMI mean = 27.12± 4.6, 24.7±3.9, respectively. The Malay ethnic group constituted considerably greater in the control participants than in the CAD group. This might indicate the reason for the high levels of TC and LDL concentrations in the control group.

**Genotype analysis:** The apoE genotype distributions among the CAD patients and the controls are shown in Table 3. *E3E4* genotype and *E4* allele were more frequent among CAD patients than the control group. *E2* allele was more frequent in the control group. However, none of the apoE genotypes or alleles frequencies had a significant association with CAD.

The one way ANOVA test analysis of each of the lipoproteins mean level distribution among the different genotypes of apoE has shown a significant association between LDL level and different genotypes of apoE as shown in Table 4. The results of Tukey *post hoc* analysis demonstrated that LDL level was significantly lower in *E2E4* genotype in comparison with *E3E3* and *E3E4* genotypes ($p = 0.039$, $p = 0.012$), respectively. The TC level was considerably higher among the *E2E4* genotype carriers compared with *E3E4* genotypes ($p = 0.031$). The TG level was insignificantly high among *E2E2* genotype carriers than among other genotypes ($p = 0.127$).

**Logistic regression model:** Logistic regression model was used to study the predictability of multiple variables and their interactions in developing CAD. This model was 88% accurate in predicting the chances to develop CAD. After controlling the confounding factors in regression analysis, the significant predictors for CAD were *E3E4* genotype, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), male gender and age with odds ratios displayed in Table 5.

Table 3: Distribution and association of apoE genotypes and allele frequencies with CAD

| | CAD (n = 185) | | Control (n = 188) | | | | Minor allele vs. *E3* allele | | |
|---|---|---|---|---|---|---|---|---|---|
| Genotypes | No. | Frequency | No. | Frequency | p-value | Minor allele | OR | 95%CI | p-value |
| *E2E2* | 9 | 0.0486 | 9 | 0.0478 | | | | | |
| *E2E3* | 9 | 0.0486 | 16 | 0.0850 | | | | | |
| *E2E4* | 8 | 0.0432 | 7 | 0.0372 | 0.47 | *E2* | 0.88 | 0.54, 1.42 | 0.63 |
| *E3E3* | 108 | 0.5830 | 113 | 0.6010 | | | | | |
| *E3E4* | 49 | 0.2640 | 36 | 0.1910 | | | | | |
| *E4E4* | 4 | 0.0216 | 6 | 0.0320 | | | | | |
| **Alleles** | | | | | | | | | |
| *E2* | 35 | 0.0936 | 41 | 0.1096 | | | | | |
| *E3* | 274 | 0.7326 | 278 | 0.7433 | 0.49 | *E4* | 1.04 | 0.71, 1.52 | 0.36 |
| *E4* | 65 | 0.1738 | 55 | 0.1471 | | | | | |

Chi-square test, OR: Odds ratio, CI: Confidence interval, p<0.05 is taken as statistically significant at 95% confidence interval

Table 4: Distribution of lipoproteins among apoE genotypes

| | Genotypes | | | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | *E3E3* | *E2E3* | *E3E4* | *E4E4* | *E2E2* | *E2E4* | p-values |
| TC | 5.57±1.31 | 5.56±1.53 | 5.91±1.56 | 5.97±1.20 | 5.63±2.07 | 4.71±0.89 | 0.061 |
| HDL | 1.12±0.40 | 1.29±0.37 | 1.15±0.42 | 1.28±0.46 | 1.17±0.40 | 1.07±0.48 | 0.339 |
| LDL | 3.72±1.20 | 3.51±1.35 | 3.91±1.33 | 3.80±0.99 | 3.47±1.60 | 2.73±0.88 | 0.031* |
| TG | 1.61±0.86 | 1.67±0.78 | 1.80±1.06 | 1.95±1.13 | 2.18±1.75 | 1.99±1.37 | 0.127 |

Mean values±SEM, *Significant difference, p<0.05 is taken as statically significant at 95% confident interval

Table 5: Results of logistic regression analysis between CAD patients and the control subjects

| Risk factors | p-values | OR | 95% CI |
|---|---|---|---|
| *E2E3* | 0.941 | 1.062 | 0.216, 5.232 |
| *E3E4* | 0.003* | 4.638 | 1.695, 12.691 |
| *E4E4* | 0.535 | 2.065 | 0.210, 20.335 |
| *E2E2* | 0.789 | 0.770 | 0.113, 5.238 |
| *E4E4* | 0.157 | 0.189 | 0.019, 1.899 |
| TG | 0.064 | 13.149 | 0.858, 201.570 |
| BMI | 0.880 | 1.007 | 0.924, 1.096 |
| SBP | 0.002* | 1.077 | 1.027, 1.130 |
| DBP | 0.000* | 0.886 | 0.830, 0.945 |
| TC | 0.183 | 0.018 | 0.000, 6.693 |
| HDL | 0.604 | 4.806 | 0.013, 1812.940 |
| LDL | 0.282 | 25.191 | 0.071, 8974.998 |
| Male | 0.000* | 6.802 | 2.825, 16.380 |
| Age | 0.000* | 1.226 | 1.157, 1.300 |

*Significant difference, p< 0.05 is taken as statically significant at 95% confident interval, TG: Triglyceride, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TC: Total cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein

**Meta-analysis:** In the analysis, the odds ratios were calculated based on *E2* or *E4* allele frequencies in comparison with the frequencies of *E3* allele in CAD patients and control groups. In both models of analysis, the random mode was selected due to the high heterogeneity among the studies. After calculation, the odds ratios were pooled together in the analysis as shown in Fig. 1 and 2. In the *E2* allele model of meta-analysis, there was no significant association between *E2* allele and CAD [p = 0.79; OR = 1.04 (0.81, 1.33) CI = 95%; $I^2$ = 71%] (Fig. 1). The pooled data showed marked heterogeneity (71%) that is mainly attributed to studies of Baum *et al.*[16], Wei *et al.*[12] and Eto *et al.*[18]. A sensitivity analysis had shown that none of the studies had imposed any undue influences to the analysis.

While in the *E4* allele model of meta-analysis, there was a significant more risk to develop CAD among *E4* carriers [p<0.0001, OR = 1.51 (1.24, 1.83) CI = 95%, $I^2$ = 68%] (Fig. 2). Similarly, the pooled data showed marked heterogeneity (68%) that is mainly attributed to studies of Wei *et al.*[12] and Kim *et al.*[20]. Similarly, the sensitivity analysis had demonstrated that none of the studies had imposed any undue influences on the analysis.

## DISCUSSION

Coronary artery disease is a chronic progressive disorder determined by the complex interaction between genetic and environmental risk factors. Several clinical trials had tried to control some the conventional risk factors for CAD such as diabetes, hypertension and smoking; however, there was a moderate reduction in the disease risk or its complications[34]. The failure to control these factor might be rather due to the influence of genetic risk factors[3]. A number of these genetic risk factors are found to act independently without determining the influence of conventional risk factors[3].

The underlying mechanism of CAD includes vascular inflammation, atherosclerosis, hypertrophy and spasm of smooth muscles within the walls of coronary arteries[35]. These changes are aggravated by dyslipidemias[36], oxidative

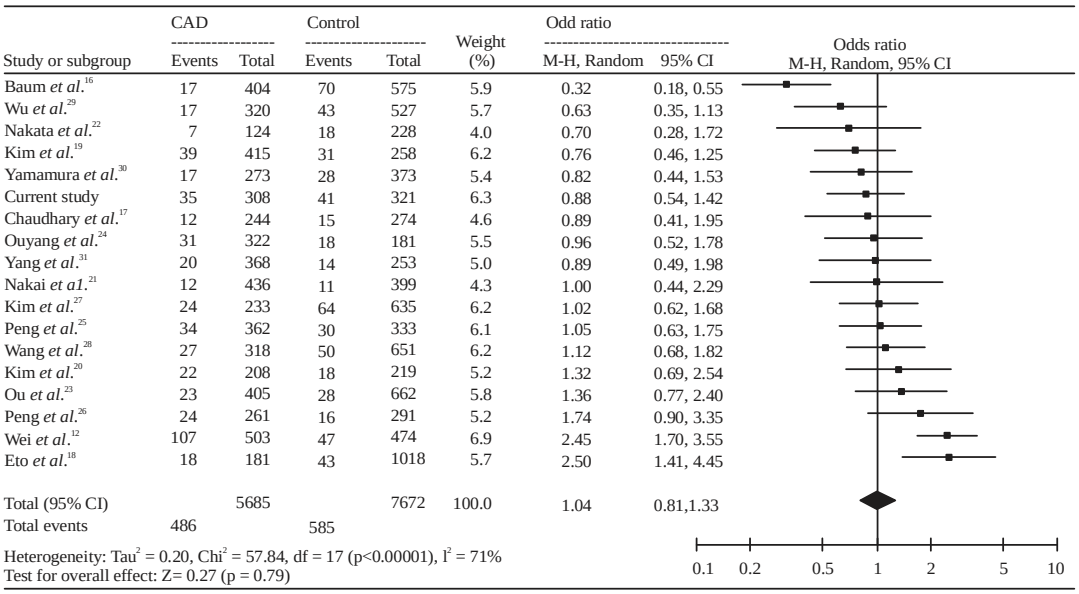


Fig. 1: Forest plot of the effect estimate for *E2* allele carriers' vs *E3* allele carriers of apoE gene polymorphism among CAD patients and controls

Meta-analysis of the combined sub-groups data showed OR = 1.04 (0.81, 1.33, 95% CI), p = 0.79, $I^2$ = 71% (Meta-analysis by Review Manager Software; version 5.3)

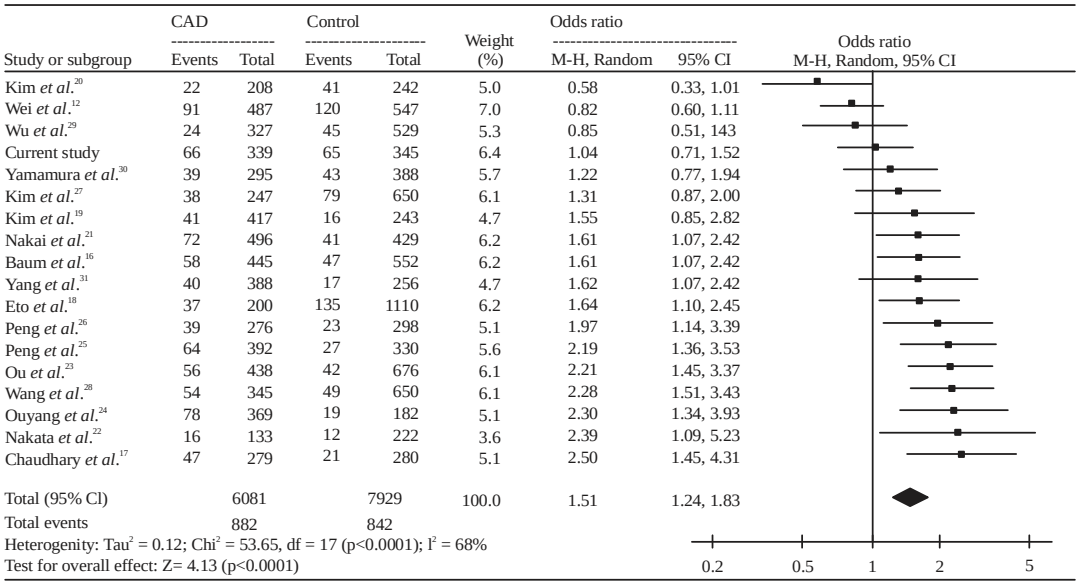


Fig. 2: Forest plot of the effect estimate for *E4* allele carriers' vs *E3* allele carriers of apoE gene polymorphism among CAD patients and controls

Meta-analysis of the combined sub-groups data showed OR = 1.51 (1.24, 1.83, 95% CI), p<0.0001, $I^2$ = 68% (Meta-analysis by Review Manager Software; version 5.3)

stress[37] and endothelial dysfunction[38]. The effect of hypercholesterolemia and oxidation prompts the deposition of LDL in the sub-endothelial space. These changes will impair the nitric oxide-dependent relaxation due to the uncoupling of nitric oxide. When the process continues, oxidation accelerates with inflammatory responses that will end up with scarring and narrowing of the coronary arteries[35].

The apoE has a recognized role in repairing most of the changes mentioned earlier. It acts as a ligand for lipoprotein metabolism enhancing the reversed clearance of vascular cholesterols[10]. Also, it processes a potent antioxidant effect that helps in maintaining the endothelial function, inhibiting vascular smooth muscles proliferation and spasm[10] and exhibits a recognized anti-inflammatory, anti-platelet

aggregation effects with nitric oxide (NO)-generating properties[10,39]. Many functional studies were conducted to focus on the role of apoE through the use of apoE knockout mice. These mice were shown to have hypercholesterolemia and had significant risk to develop coronary arteries occlusion, myocardial infarction and premature death[40]. Other studies noted that the absence of apoE in mice correlates with the appearance of myocardial infarction markers[41] and vascular oxidation stress changes[42].

In the present case-control study, the genotypes distribution among the control participants was in agreement with Hardy-Weinberg equilibrium. The ethnic distribution of apoE genotypes and alleles in the control group was consistent with one Malaysian study[12]. Also in this study, the *E3* allele was present in the majority of participants, followed by the *E4* and *E2* alleles, respectively.

Although there was a significant ethnicity, gender and age differences between the CAD patients and the control participants; however further analysis has shown neither gender nor the ethnicity or age has any significant association with the apoE genotypes distribution (p = 0.93, 0.8 and 0.935, respectively).

The apoE genotypes and alleles were not significantly associated with CAD although carriers of *E4* allele had shown a slightly increased risk for developing CAD while *E2* allele had a lower risk for CAD [OR =1.04 (0.71, 1.52 95% CI), 0.88 (0.54, 1.42 95% CI)] respectively. In the logistic regression model analysis, the *E3E4* genotypes in comparison with *E3E3* genotype, men compared with women had a significant more dependent probability for developing CAD [OR 4.638 (1.695, 12.691 95% CI) or 6.802 (2.825, 16.38 95% CI)], respectively. Also, an increase of age and blood pressure reading had a significant more dependent probability for developing CAD as shown in Table 5.

In the meta-analysis, the *E2* allele was not significantly associated with CAD (p-value = 0.79). The findings from included studies regarding this association were incongruent, in which some studies elucidated a reduced risk for CAD among *E2* allele carriers, while other studies demonstrated an increased risk for CAD among *E2* allele carriers. Wei *et al.*[12] and Eto *et al.*[18] demonstrated a significant association between *E2* allele and CAD, OR = 2.45 (1.7, 3.55 95% CI), 2.5 (1.41, 4.45), respectively. While Baum *et al.*[16] had detected a significantly reduced risk towards CAD among carriers of *E2* allele (p-value = 0.0001). Results from these studies caused the considerable heterogeneity in the meta-analysis as shown in Fig. 1.

In the meta-analysis, the *E4* allele was significantly associated with CAD (p-value<0.0001). The majority of the studies were in line with this finding; however, Kim *et al.*[19], Wei *et al.*[12] and Wu *et al.*[28] elucidated a non-significant association between *E4* allele and CAD (p-value>0.05). The variability was unlikely to be due to any publication bias as indicated by the symmetrical funnels plot. Further subgroup meta-analysis was conducted after stratifying the studies by their countries. However, all of the groups had shown a high degree of heterogeneity ($I^2$ value>50%).

The findings in the original study did not demonstrate the significant association between *E4* allele and CAD probably due to the small sample size. Therefore, large-scale studies with the special concern of controlling other risk factors for CAD are recommended. Factors such as ethnicity, obesity, age and lifestyle and history of smoking and diseases such as hypertension and diabetes are major contributors to the development of CAD. Controlling these factors in the patients and the controls group would provide a more precise description of the association between apoE gene polymorphism and CAD.

The meta-analysis had overcome the problem of small sample size and had remarkably improved the precision of the study. However, the unavoidable weakness in the analysis is related to the heterogeneity among the included studies. The heterogeneity is mainly attributed to a minority of the studies as shown in Fig. 2 that might be related to different recruitment protocols.

Therefore, apoE genotyping might be necessary particularly when associated with other risk factors such as high blood pressure or among individuals with apparent family history of CAD. Aggressive modifications of environmental risk factors among these susceptible individuals (carriers of *E4* allele) might protect them from developing coronary artery disease and/or improve the prognosis among the patients.

## CONCLUSION

This study provides some evidence about the increased risk to develop CAD among people with *E4* allele. This risk is much higher when it involves male gender with an increase of age and elevated blood pressure.

## SIGNIFICANCE STATEMENTS

This study highlights the role of apoE gene polymorphism as a genetic risk factor for coronary artery disease among Southeast Asia and East Asia populations. The original study elucidated the significant risk to develop CAD among carriers of apoE epsilon 4 especially when associated with high blood

pressure. The meta-analysis provided an additional support by showing the independent significant risk of apoE epsilon 4 among populations in East Asian and South East Asian region. The overall finding exhibits the important interplay between apoE gene polymorphism and the environmental factors that might contribute to the risk of coronary artery disease.

## ACKNOWLEDGMENT

This study was supported by the research initiative grant scheme from the International Islamic University Malaysia (RIGS 15-089-0089), Kuantan, Pahang, Malaysia.

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