Atherosclerosis 232 (2014) 180–185

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

APOE polymorphism and carotid atherosclerosis in Korean population: The Dong-gu Study and the Namwon Study

Min-Ho Shin [a,i], Jin-Su Choi [a], Jung-Ae Rhee [a], Young-Hoon Lee [c], Hae-Sung Nam [d], Seul-Ki Jeong [e], Kyeong-Soo Park [f], Hye-Yeon Kim [f], So-Yeon Ryu [g], Seong-Woo Choi [g], Hee Nam Kim [h], Hye-Rim Song [a], Jane A. Cauley [i], Sun-Seog Kweon [a,b,*]

[a] Department of Preventive Medicine, Chonnam National University Medical School, Gwangju, Republic of Korea
[b] Jeonnam Regional Cancer Center, Chonnam National University Hwasun, Hwasun Hospital, Republic of Korea
[c] Department of Preventive Medicine & Institute of Wonkwang Medical Science, Wonkwang University College of Medicine, Iksan, Republic of Korea
[d] Department of Preventive Medicine, Chungnam National University Medical School, Daejeon, Republic of Korea
[e] Department of Neurology & Research Institute of Clinical Medicine, Chonbuk National University, Biomedical Institute of Chonbuk National University Hospital, Jeonju, Republic of Korea
[f] Department of Preventive Medicine, Seonam University College of Medicine, Namwon, Republic of Korea
[g] Department of Preventive Medicine, Chosun University Medical School, Gwangju, Republic of Korea
[h] Genome Research Center for Hematopoietic Diseases, Chonnam National University Hwasun Hospital, Hwasun, Jeollanam-do, Republic of Korea
[i] Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, USA

ARTICLE INFO

Article history:
Received 20 March 2013
Received in revised form
14 October 2013
Accepted 1 November 2013
Available online 20 November 2013

Keywords:
Apolipoprotein E
Polymorphism
Carotid artery plaque
Intima-media thickness

ABSTRACT

Objective: We evaluated the association between *APOE* polymorphism and carotid atherosclerosis in two large independent cohorts from South Korea.
Methods: The datasets were from the Dong-gu Study ($N = 9056$) and the Namwon Study ($N = 10{,}158$). Carotid ultrasonography was performed to measure carotid intima-media thickness (IMT) and the presence of carotid plaques. The *APOE* polymorphism was determined by PCR-RFLP. We performed combined and separate analyses for the two datasets.
Results: In the combined analysis, individuals with *E2E2* or *E2E3* genotype had a lower common carotid IMT compared with individuals with *E3E3* genotype (0.684 mm vs. 0.736 mm, $p = 0.007$; 0.718 mm vs. 0.736 mm, $p < 0.001$, respectively). This association was very slightly attenuated but remained statistically significant after adjustment for blood lipids (0.690 mm vs. 0.736 mm, $p = 0.033$; 0.725 mm vs. 0.736 mm, $p = 0.005$, respectively). Compared with individuals with *E3E3* genotype, individuals with *E2E3* genotype had lower risk for carotid plaque (odds ratio (OR) = 0.83, 95% confidence interval (CI) = 0.75–0.93), while individuals with *E3E4* genotype had a higher risk for carotid plaque (OR = 1.09, 95% CI = 1.00–1.20). After adjustment for blood lipids, ORs of *E2E3* genotype for carotid plaque was slightly attenuated but remained significant (OR = 0.87 95% CI = 0.78–0.97), while OR of *E3E4* genotype were slightly attenuated and not significant (OR = 1.08, 95% CI, 0.99–1.18).
Conclusions: We found that *APOE* polymorphism is associated with carotid atherosclerosis and this association was partly mediated through blood lipid. Our results suggest that *APOE* polymorphism may influence atherosclerosis through non-lipid pathways.

1. Introduction

Apolipoprotein E (apoE = protein, *APOE* = gene) binds to receptors on the liver to help mediate clearance of triglyceride-rich lipoproteins from the plasma [1–3]. The *APOE* gene has three common alleles (*E2*, *E3* and *E4*) arising from two single nucleotide polymorphisms in exon 4, yielding six possible genotypes (*E2E2*, *E2E3*, *E2E4*, *E3E3*, *E3E4 and E4E4*) [4]. *E2* allele is associated with lower LDL-C levels, and *E4* allele with higher LDL-C levels, compared

* Corresponding author. Jeonnam Regional Cancer Center, Chonnam National University Hwasun Hospital, 160 Ilsim-ri, Hwasun-eup, Hwasun-gun, Jeonnam 519-763, Republic of Korea. Tel.: +82 61 379 7723; fax: +82 61 379 7880.
E-mail address: ujingogo@paran.com (S.-S. Kweon).

0021-9150/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.atherosclerosis.2013.11.027

with *E3* allele [5]. Approximately 7% of the variation in total cholesterol levels is due to *APOE* genotypes [4]. Therefore, *APOE* genotypes would be expected to confer a risk of atherosclerotic disease.

The association between carotid atherosclerosis and *APOE* genotypes has been examined in the general population. However, the results of these studies have been inconsistent. Some studies [6–13] found significant associations, whereas others [14–17] did not. Many studies were not adequately powered to assess whether *APOE* genotypes is associated with carotid atherosclerosis. In particular, there have been no studies involving Asian populations with over 500 subjects.

Therefore, we evaluated the association between *APOE* polymorphism and carotid atherosclerosis in two large independent cohorts from South Korea: the Dong-gu Study and the Namwon Study.

2. Methods

2.1. Subjects

The Dong-gu Study and the Namwon Study are ongoing prospective studies designed to investigate the prevalence, incidence, and risk factors for chronic disease in urban and rural populations, respectively. Details of the study subjects and measurements have been published previously [18]. In the Dong-gu Study, 9260 subjects (50 years of age and older) from the Dong-gu district of Gwangju Metropolitan City in South Korea were enrolled from 2007 to 2010. Of these, 204 subjects were excluded because of missing data on carotid ultrasonography, genotype, blood lipids and lifestyle. After these exclusions, data on 9056 participants (3625 men and 5431 women) were included in the present analyses. In the Namwon Study, 10,667 subjects (4201 men and 6466 women) aged 45–74 years from Namwon City, Jeollabuk-do Province, South Korea, were enrolled from 2004 to 2007. Of these, 522 subjects were excluded because of missing data on carotid ultrasonography, genotype, blood lipids and lifestyle. With these exclusions, data on 10,145 participants (4002 men and 6143 women) were included in the present analyses.

These two studies were approved by the Institutional Review Board of Chonnam National University Hospital, and informed consent was obtained from each subject.

2.2. APOE genotyping

Genomic DNA was extracted from peripheral blood with the AccuPrep Genomic DNA Extraction Kit (Bioneer, Seoul, Korea) or the QIAamp DNA Mini Kit (Qiagen Inc., Chatsworth, CA, USA) according to the manufacturer's protocol. *APOE* genotypes were determined as described by Hixson and Vernier, with slight modification [19]. Our *APOE* genotyping method has been reported previously [20].

2.3. Carotid ultrasound

Carotid atherosclerosis was assessed by physicians using high-resolution mode B ultrasonographic scans of the carotid arteries (SONOACE 9900, Medison, Korea) with an electrical linear array transducer (7.5 MHz). Images of the common carotid artery (CCA), carotid bulb, and internal carotid artery were used to evaluate intima-media thickness (IMT) and plaque. A single trained reader analyzed the frozen images using SigmaScan Pro Version 5.0.0 (SPSS Inc., Chicago, IL, USA) according to a standardized protocol. The Common carotid artery IMT (CCA-IMT) was determined as the average of the maximum IMT of the left and right common carotid arteries. The reader also assessed the presence of carotid plaques, defined as focal structures that encroached into the lumen by at least 100% of the surrounding IMT value. Sonographers and the carotid reader were blind to genotypes and other information about the subjects. The presence of carotid plaques was recorded if at least one lesion was detected in any segment in both carotid arteries.

2.4. Other clinical variables

Information on each subject's medical history and lifestyle was obtained using standardized questionnaires. Smoking status was classified as non-smoker, former smoker, or current smoker. Height and weight were measured to the nearest 0.1 cm, and weight to the nearest 0.1 kg. Body mass index was calculated by dividing weight (in kilograms) by height squared (in meters squared).

Diabetes was defined by a fasting plasma glucose $\geq$126 mg/dl or use of insulin or hypoglycemic medication. Blood pressure was measured in the right upper arm using a mercury sphygmomanometer (Baumanometer; WA Baum Co., Inc., Copiague, NY, USA) with an appropriately sized cuff after subjects rested at least 5 min while seated. Three consecutive measurements of systolic and diastolic blood pressures were performed at 1-min intervals, and the average was used in the analysis. Hypertension was defined by systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg, or use of blood pressure-lowering drugs.

Blood samples were drawn from an antecubital vein in the morning following a 12-h overnight fast. Serum was separated within 30 min of collection and stored at -70 °C until analyzed. Serum total cholesterol, high-density lipoprotein-cholesterol (HDL-cholesterol), triglycerides, and fasting blood glucose levels were measured using enzymatic methods. Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald's formula in cases where triglyceride levels were $<$400 mg/dl [21]. All samples were analyzed using an automatic analyzer (model 7600 chemical analyzer; Hitachi Ltd., Tokyo, Japan).

2.5. Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD) or percentage for categorical variables. Analysis of variance and Pearson's chi-square test were used to compare baseline characteristics across *APOE* genotypes. Because triglyceride levels were not normally distributed, log-transformed values were used for statistical comparison. We performed both genotype-based and allele-based analyses for *APOE* polymorphism. The *APOE* polymorphism was categorized into *E2E2*, *E2E3*, *E2E4*, *E3E3*, *E3E4* or *E4E4* for genotype-based analyses and categorized into *E2* (*E2E2* and *E2E3*), *E3* (*E3E3*), or *E4* (*E3E4* and *E4E4*) for allele-based analyses. Subjects with the *E2E4* genotype were excluded because of potential opposing biological effects of the *E2* and *E4* alleles. We performed separate analyses as well as a combined analysis for the two studies to increase the statistical power, as there was no statistically significant heterogeneity for the results in each study group. Multiple linear regression models were used to evaluate the association between *APOE* genotypes and IMT and multiple logistic regression models were used to evaluate the association between *APOE* genotypes and carotid plaque. Model I was adjusted for age, sex, BMI, smoking, diabetes and hypertension. Model II was further adjusted for total cholesterol, HDL cholesterol, and log-transformed triglycerides levels to evaluate whether associations were mediated by blood lipids. We also assessed for interaction of *APOE* genotypes with age, sex, and smoking status in full adjusted multivariable models to examine whether these covariates modified the association between *APOE* genotypes and carotid atherosclerosis. In the combined analysis, we further adjusted for the study group in all models. Hardy–Weinberg equilibrium was

tested by use of a chi-square goodness of fit test. Statistical analyses were performed using SPSS version 20.0 (SPSS, Inc., an IBM Company, Chicago, Illinois, USA).

3. Results

The characteristics of both study cohorts according to *APOE* genotypes are presented in Table 1. The overall mean age was younger in the Namwon Study than in the Dong-gu Study (61.6 ± 7.8 years vs. 65.2 ± 8.2 years). The *APOE* genotypes frequencies were consistent with Hardy–Weinberg equilibrium ($p = 0.94$ for the Dong-gu Study, $p = 0.84$ for the Namwon Study). The frequency of *APOE* genotypes for *E2E2*, *E2E3*, *E2E4*, *E3E3*, *E3E4*, and *E4E4* was 0.4, 10.4, 1.2, 72.0, 15.1, and 0.9%, respectively in the combined cohort. The *APOE* allele frequency for *E2*, *E3*, and *E4* was 6.2, 84.8, and 9.0%, respectively in combined cohort. The allele frequency in this study was similar to that from other Korean [22,23] and Japanese [24] populations. The frequencies of *E2* and *E4* allele in this study were lower than in Caucasians and African Americans [10,12,25]. There were statistically significant differences in total cholesterol, HDL cholesterol, triglyceride, and LDL cholesterol levels across the *APOE* genotypes and alleles in both cohorts. No other statistically significant differences were observed.

The associations of *APOE* genotypes and allele with CCA-IMT are also shown in Table 2. There were statistically significant differences in CCA-IMT across the *APOE* genotypes and alleles in each cohorts and in the combined analysis in all models. In the genotype-based analysis, compared with the *E3E3* group, individuals with *E2E2* or *E2E3* genotype had a lower IMT in the Dong-gu Study cohort (0.653 mm vs. 0.725 mm, $p = 0.014$; 0.708 mm vs. 0.725 mm, $p = 0.004$, respectively) and individuals with *E2E3* genotype only had a lower IMT in the Namwon Study cohort (0.726 mm vs. 0.746 mm, $p < 0.001$). In the combined analysis of both studies, individuals with *E2E2* or *E2E3* genotype had a lower CCA-IMT compared with the *E3E3* group (0.684 mm vs. 0.736 mm, $p = 0.007$; 0.718 mm vs. 0.736 mm, $p < 0.001$, respectively). In the allele-based analysis, compared with the *E3* allele group, carriers of *E2* allele had a lower CCA-IMT in the Dong-gu Study, the Namwon Study, and the combined analysis (0.706 mm vs. 0.725 mm, $p < 0.001$; 0.726 mm vs. 0.746 mm, $p < 0.001$; 0.716 mm vs. 0.736 mm, $p < 0.001$, respectively) whereas the *E4* allele showed no relationship. These associations were slightly attenuated but remained statistically significant after adjustment for blood lipids in combined analysis. The relationship between *APOE* genotypes and carotid plaque are shown in Table 3. In the genotype-based analysis, individuals with *E2E3* genotype had a lower risk for carotid plaque than the *E3E3* genotype in the Dong-gu Study, the Namwon Study and the combined analysis (OR = 0.85, 95% CI = 0.73–0.99; OR = 0.80, 95% CI = 0.69–0.93; OR = 0.83, 95% CI = 0.74–0.92, respectively), while individuals with *E3E4* genotype had higher risk for carotid plaque than the *E3E3* group (OR = 1.09, 95% CI = 1.00–1.20) in the combined analysis. In the allele-based analysis, compared with the *E3* allele group, carriers of *E2* allele had lower risk for carotid plaque than *E3* allele group in the Dong-gu Study, the Namwon Study and combined analysis (OR = 0.85, 95% CI = 0.74–0.99; OR = 0.81, 95% CI = 0.69–0.95; OR = 0.84, 95% CI = 0.74–0.93, respectively), whereas carriers of *E4* allele had a higher risk for carotid plaque than the *E3* allele group (OR = 1.10, 95% CI = 1.01–1.12) in combined analysis. After adjustment for blood lipids, ORs of *E2E3* genotype or *E2* allele for carotid plaque were slightly attenuated but remained statistically significant (OR = 0.87 95% CI = 0.78–0.97; OR = 0.88, 95% CI = 0.79–0.98, respectively), whereas ORs of *E3E4* genotype and *E4* allele were slightly attenuated and not statistically significant (OR = 1.08, 95% CI, 0.99–1.18; OR = 1.08, 95% CI = 0.99–1.18, respectively) in the combined analysis.

Table 1
Characteristics of the study subjects according to *APOE* genotype.

	E2E2	*E2E3*	*E2E4*	*E3E3*	*E3E4*	*E4E4*	*p*-value
Dong-gu Study							
N	40	975	118	6426	1413	84	
Age (years)	62.6 ± 7.2	65.0 ± 8.1	64.0 ± 7.9	65.3 ± 8.2	65.2 ± 8	64.4 ± 8.3	0.142
Men (%)	14 (35.0)	411 (42.2)	43 (36.4)	2548 (39.7)	574 (40.6)	35 (41.7)	0.613
Body mass index (kg/m^2)	24.5 ± 2.4	24.3 ± 2.9	24.2 ± 3	24.4 ± 3	24.2 ± 2.9	24.1 ± 2.8	0.436
Current smoking (%)	4 (10.0)	109 (11.2)	17 (14.4)	702 (10.9)	154 (10.9)	6 (7.1)	0.732
Hypertension (%)	13 (32.5)	441 (45.2)	59 (50.0)	2863 (44.6)	641 (45.4)	35 (41.7)	0.483
Diabetes mellitus (%)	12 (30.0)	201 (20.6)	21 (17.8)	1218 (19.0)	261 (18.5)	15 (17.9)	0.393
Myocardial infarction (%)	2 (5.0)	8 (0.8)	0 (0.0)	90 (1.4)	19 (1.3)	0 (0.0)	0.105
Stroke (%)	1 (2.5)	34 (3.5)	9 (7.6)	258 (4.0)	62 (4.4)	3 (3.6)	0.368
Total cholesterol (mg/dl)	192.5 ± 61.1	188.9 ± 37.9*	195.5 ± 44.4	202.9 ± 39.6	203.2 ± 40.6	200.0 ± 41.6	<0.001
HDL cholesterol (mg/dl)	54.2 ± 13.2	52.5 ± 12.2	51.3 ± 14.3	51.8 ± 11.9	50.0 ± 11.7*	47.7 ± 12*	<0.001
Triglyceride (mg/dl)	195.8 ± 145.9*	151.8 ± 121.6*	164.1 ± 127.7	139.2 ± 96.7	148.8 ± 96.5*	171.4 ± 103.5*	<0.001
LDL cholesterol (mg/dl)[a]	90.4 ± 37.9*	107.5 ± 31.8*	114.5 ± 39.5*	124.4 ± 35.3	124.9 ± 37.1	120 ± 39.3	<0.001
Namwon Study							
N	36	1029	107	7401	1484	88	
Age (years)	59.0 ± 7.1	61.7 ± 7.9	63.1 ± 7.8	61.6 ± 7.8	61.4 ± 7.8	61.5 ± 6.7	0.090
Men (%)	16 (44.4)	403 (39.2)	31 (29.0)	2943 (39.8)	573 (38.6)	36 (40.9)	0.292
Body mass index (kg/m^2)	23.9 ± 2.8	24.2 ± 3.0	24.5 ± 3.1	24.4 ± 3.1	24.4 ± 3.1	24.0 ± 3.2	0.261
Current smoking (%)	5 (13.9)	173 (16.8)	14 (13.1)	1141 (15.4)	223 (15.0)	11 (12.5)	0.733
Hypertension (%)	9 (25.0)	383 (37.2)	36 (33.6)	2975 (40.2)	586 (39.5)	35 (39.8)	0.139
Diabetes mellitus (%)	2 (5.6)	118 (11.5)	10 (9.3)	922 (12.5)	174 (11.7)	14 (15.9)	0.453
Myocardial infarction (%)	0 (0.0)	2 (0.2)	0 (0.0)	30 (0.4)	5 (0.3)	1 (1.1)	0.693
Stroke (%)	3 (8.3)	32 (3.1)	2 (1.9)	281 (3.8)	45 (3.0)	4 (4.5)	0.273
Total cholesterol (mg/dl)	175.8 ± 57.9	176.7 ± 36.7*	179.3 ± 33.7*	190.6 ± 36.7	192.7 ± 37.1	193.8 ± 41.4	<0.001
HDL cholesterol (mg/dl)	43.0 ± 10.5	49.0 ± 13.0*	47.1 ± 12.9	47.8 ± 11.9	45.7 ± 11.4*	43.9 ± 10.5*	<0.001
Triglyceride (mg/dl)	204.1 ± 150.7	167.3 ± 129.5*	181.1 ± 112	153.6 ± 105.2	168.7 ± 134.1*	199.1 ± 154.2*	<0.001
LDL cholesterol (mg/dl)[a]	88.4 ± 38.8*	95.8 ± 29.1*	96.9 ± 30.1*	113.7 ± 32.6	116.0 ± 33.5	113.9 ± 34.4	<0.001

Values are mean ± SD or number (percentage).
HDL, high-density lipoprotein.
*$P < 0.05$ compared with *E3E3* with a Bonferroni multiple comparisons test.
[a] LDL cholesterol was calculated from Friedewald's formula if triglyceride levels were <400 mg/dl.

Table 2
Association of *APOE* genotype and allele with common carotid intima-media thickness.

Genotype	Dong-gu Study				Namwon Study				Combined cohort			
	Model I	p^*	Model II	p^*	Model I	p^*	Model II	p^*	Model I	p^*	Model II	p^*
E2E2	0.653 ± 0.022	0.014	0.663 ± 0.022	0.068	0.716 ± 0.021	1.000	0.718 ± 0.020	1.000	0.684 ± 0.015	0.007	0.690 ± 0.015	0.033
E2E3	0.708 ± 0.004	0.004	0.714 ± 0.004	0.347	0.726 ± 0.004	<0.001	0.734 ± 0.004	0.068	0.718 ± 0.003	<0.001	0.725 ± 0.003	0.005
E2E4	0.730 ± 0.013	1.000	0.732 ± 0.013	1.000	0.726 ± 0.012	1.000	0.733 ± 0.012	1.000	0.729 ± 0.009	1.000	0.734 ± 0.009	1.000
E3E3	0.725 ± 0.002	–	0.725 ± 0.002	–	0.746 ± 0.001	–	0.746 ± 0.001	–	0.736 ± 0.001	–	0.736 ± 0.001	–
E3E4	0.729 ± 0.004	1.000	0.727 ± 0.004	1.000	0.744 ± 0.003	1.000	0.740 ± 0.003	1.000	0.737 ± 0.002	1.000	0.734 ± 0.002	1.000
E4E4	0.728 ± 0.015	1.000	0.726 ± 0.015	1.000	0.762 ± 0.013	1.000	0.757 ± 0.013	1.000	0.746 ± 0.01	1.000	0.743 ± 0.01	1.000
p-value	<0.001		0.013		<0.001		0.025		<0.001		<0.001	
E2	0.706 ± 0.004	<0.001	0.712 ± 0.004	0.018	0.726 ± 0.004	<0.001	0.734 ± 0.004	0.007	0.716 ± 0.003	<0.001	0.723 ± 0.003	<0.001
E3	0.725 ± 0.002	–	0.725 ± 0.002	–	0.746 ± 0.001	–	0.746 ± 0.001	–	0.736 ± 0.001	–	0.736 ± 0.001	–
E4	0.730 ± 0.004	0.870	0.727 ± 0.004	1.000	0.745 ± 0.003	1.000	0.741 ± 0.003	0.575	0.738 ± 0.002	1.000	0.735 ± 0.002	1.000
p-value	<0.001		0.012		<0.001		0.007		<0.001		<0.001	

Data are presented as mean ± standard error.
Model I, adjusted age, sex, BMI, smoking, diabetes and hypertension.
Model II, further adjusted for total cholesterol, HDL cholesterol, and log-transformed triglycerides.
In combined cohort, further adjusted for study group in all models.
In combined cohort, further adjusted for study group in all models.
*P-value when compared with *E3E3* or E3 with a Bonferroni multiple comparisons test.

There was no effect modification of sex, obesity, smoking, hypertension, diabetes mellitus, or total cholesterol on the association of *APOE* genotypes with CCA-IMT and plaque (data not shown).

4. Discussion

In these two large population-based studies, we found that individuals with *E2E2* and *E2E3* genotypes had lower carotid IMT compared with those with *E3E3* genotype. We also found that individuals with *E2E3* genotype was associated with lower risk of carotid plaque, whereas individuals with *E3E4* genotype was associated with higher risk of carotid plaque compared with *E3E3* genotype. After adjusting for blood lipids, these associations were slightly attenuated which suggested that the association between *APOE* genotypes and carotid atherosclerosis is partly mediated through blood lipids. Although our results are not a novel finding and have been reported previously, to our knowledge, this is the largest population-based study to date and the first study to investigate the association of *APOE* gene polymorphisms and carotid atherosclerosis in an Asian population with over 500 participants; this enabled us to confidently confirm the small but significant effect of genotype.

In our study, individuals with *E2E2* or *E2E3* genotype had lower CCA-IMT than individuals with *E3E3* genotype, but *E4* allele had no effect. One meta-analysis of 22 published studies showed a statistically significant difference of 0.046 mm (95% CI 0.029–0.062) in CCA-IMT for *E4* vs. *E2* allele, but in analyses restricted to studies of >1000 subjects, the mean difference was reduced to 0.017 mm (95% CI 0.012–0.023) [26]. Considering the sample size, our study showing the difference between *E2* and *E4* of 0.021 mm (95% CI 0.013–0.027) (data not shown) was in agreement with this meta-analysis. In addition, this observed effect size was similar in magnitude to that of current smoking (0.023 mm, 95% CI 0.017–0.029) and hypertension (0.024 mm 95% CI 0.020–0.028) (data not shown). There have been five studies [8,10–13] which enrolled more than 1000 subjects. Of these, three studies [8,10,12] found an association between the *E2* allele and lower IMT and two studies [8,27] found an association between the E4 allele and higher IMT. In the Rotterdam Study of 5401 participants [28], individuals with the *E2E3* genotype had lower CCA-IMT than those with *E3E3* genotype. In the Framingham Study of 2723 participants [9], carriers of the *E2* allele had lower CCA-IMT than carriers of the *E4* allele in women only. In the ARIC study of 12,491 participants [29], compared with carriers of the *E3* allele, carrier of the *E2* allele had lower carotid IMT and carriers of *E4* allele had higher carotid IMT in both Caucasian and African Americans, but, after adjusting for lipid parameters, only carriers of the *E4* allele had higher carotid IMT in African Americans. In the Three-City Study of 5856 participants [8], individuals with *E3E4* had higher CCA-IMT than those with *E3E3* genotype. However, in the Carotid Ultrasound Disease Assessment Study (CUDAS) [4], there were no statistically significant independent associations between *APOE* genotypes and carotid IMT.

In our study, individuals with *E2E3* or *E2E2* genotype had a lower risk for carotid plaque compared with the E3E3 group,

Table 3
Odds ratio and 95% confidence interval of *APOE* genotype and allele for carotid plaque.

	Dong-gu Study		Namwon Study		Combined cohort	
	Model I	Model II	Model I	Model II	Model I	Model II
E2E2	1.00 (0.50–1.99)	1.04 (0.52–2.08)	1.24 (0.59–2.60)	1.24 (0.59–2.62)	1.09 (0.66–1.82)	1.12 (0.67–1.86)
E2E3	0.85 (0.73–0.99)	0.89 (0.77–1.04)	0.80 (0.69–0.93)	0.83 (0.71–0.98)	0.83 (0.74–0.92)	0.87 (0.78–0.97)
E2E4	0.76 (0.50–1.15)	0.78 (0.51–1.19)	0.71 (0.45–1.12)	0.73 (0.46–1.16)	0.75 (0.55–1.02)	0.77 (0.57–1.06)
E3E3	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
E3E4	1.09 (0.96–1.24)	1.08 (0.95–1.22)	1.10 (0.97–1.24)	1.08 (0.95–1.22)	1.09 (1.00–1.20)	1.08 (0.99–1.18)
E4E4	1.26 (0.79–2.00)	1.24 (0.78–1.97)	1.08 (0.68–1.73)	1.05 (0.65–1.67)	1.17 (0.84–1.62)	1.14 (0.82–1.58)
E2	0.85 (0.74–0.99)	0.90 (0.77–1.05)	0.81 (0.70–0.95)	0.85 (0.73–0.99)	0.84 (0.75–0.93)	0.88 (0.79–0.98)
E3	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
E4	1.10 (0.97–1.24)	1.09 (0.96–1.23)	1.10 (0.97–1.24)	1.07 (0.95–1.22)	1.10 (1.01–1.12)	1.08 (0.99–1.18)

Model I, adjusted age, sex, BMI, smoking, diabetes and hypertension.
Model II, further adjusted for total cholesterol, HDL cholesterol, and log-transformed triglycerides.
In combined cohort, further adjusted for study group in all models.

whereas individuals with *E3E4* genotype had higher risk. Of the five previous studies that included more than 1000 subjects [8,10–13], three studies [4,8,30] investigated the association between *APOE* polymorphism and carotid plaque. Similar to our study, the findings of the Three-City Study [8] suggested that carriers of *E2* (*E2E2*/*E2E3*) had lower risk of carotid plaque (OR = 0.79, 95% CI = 0.66–0.95), whereas individuals with *E4E4* genotype had higher risk (OR = 2.12, 95% CI = 1.27–3.53) compared with those with *E3E3* genotype. However, individuals with *E2E3* genotype only had lower risk of carotid plaque (OR = 0.6, 95% CI = 0.4–0.8) in the Rotterdam study [31] and in only women (OR, 0.40; 95% CI, 0.17–0.91) in the CUDAS study [4]. Our results are also consistent with a prior meta-analysis reporting that the OR for coronary disease was 0.80 (95% CI, 0.70–0.90) in *E2* carriers and was 1.06 (95% CI, 0.99–1.13) in *E4* carriers compared with *E3E3* [32].

The mechanisms by which *APOE* polymorphism affect carotid atherosclerosis are not fully understood. This association can be explained not only by differences in blood lipid levels among *APOE* genotype groups but also by non-lipid pathways. In our combined analysis, the CCA-IMT difference of 0.020 mm (95% CI 0.013–0.027) between *E2* allele and *E3* allele carries was slightly attenuated to 0.013 mm (95% CI 0.005–0.020) after adjustment for blood lipids, suggesting that the effects were partly mediated through blood lipids and may also be mediated through non-lipid pathways. Of the previous four studies involving more than 1000 subjects that reported the statistically significant associations between *APOE* polymorphism and IMT, three adjusted for blood lipids. In the Framingham Study, the difference in CCA-IMT between *E2* allele and *E4* allele groups decreased from 0.03 mm to 0.02 mm and remained statistically non-significant after adjustment for blood lipids. In the ARIC study, the differences in the CCA-IMT between carriers of *E3* allele and of *E2* or *E4* allele decreased from 0.011 mm to 0.005 for both alleles in Caucasians but not in African Americans. However, in the Rotterdam study, adjustments for total and HDL cholesterol did not change the association. *APOE* genotypes may also influence carotid atherosclerosis through antioxidant and inflammation modulatory properties of apoE [31]. ApoE has antioxidant activity varying in strength according to the allele (*E2* > *E3* > *E4*) [31]. ApoE is an endogenous immunomodulatory agent that can affect both the innate and the adaptive immune responses [28,30,33]. Differential inflammation modulatory properties among the apoE isoforms have also been suggested [27,29,34].

The strengths of this study included its very large sample size and adequate statistical power. It is difficult to detect any small-sized effect of *APOE* genotypes without a large enough sample size. A sample size of over 6000 subjects would be necessary for 80% power at $p < 0.05$ to detect a per-genotype mean carotid IMT difference of 0.01 mm in a co-dominant model assuming a minor allele frequency of 0.2 [35]. Additionally, we were able to correct for the problem of multiple comparisons between genotypes using the Bonferroni correction because of the very large sample size. Nevertheless, several limitations were also present. First, only a single measurement of blood lipids was used in the analysis. Single measurement for blood lipids may have low reliability in representing lifetime exposure. Therefore, the mediation effect of blood lipids when evaluating the relationship between *APOE* genotypes and carotid atherosclerosis may be underestimated. Second, we did not use ECG-gated carotid ultrasonography because the high-resolution B-mode ultrasound used in our study did not provide ECG on carotid measurements. To overcome this problem, we evaluated consecutive images of the carotid artery during a 10-s phase (video clip) and stored images of the minimum common carotid artery diameter, representing the end-diastolic phase. Third, potential differences in IMT measurements across this and previous studies may influence the inconsistent results.

In conclusion, from analyses of these two large-scale studies, we found that the *APOE* polymorphism is associated with carotid atherosclerosis and this association was partly mediated through blood lipids. Our results suggest that *APOE* polymorphism may influence atherosclerosis through non-lipid pathways.

Conflict of interest

None.

Acknowledgments

This study was supported by Research Institute of Medical Sciences, Chonnam National University. The Dong-gu Study was partially supported by the Health Promotion Fund, Ministry of Health & Welfare, Republic of Korea (07-27).

References

[1] Eichner JE, Dunn ST, Perveen G, et al. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol 2002;155:487–95.
[2] Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 1988;240:622–30.
[3] Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann Intern Med 2004;141:137–47.
[4] Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. Arteriosclerosis 1988;8:1–21.
[5] Dallongeville J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. J Lipid Res 1992;33:447–54.
[6] Cattin L, Fisicaro M, Tonizzo M, et al. Polymorphism of the apolipoprotein E gene and early carotid atherosclerosis defined by ultrasonography in asymptomatic adults. Arterioscler Thromb Vasc Biol 1997;17:91–4.
[7] Ilveskoski E, Loimaala A, Mercuri MF, et al. Apolipoprotein E polymorphism and carotid artery intima-media thickness in a random sample of middle-aged men. Atherosclerosis 2000;153:147–53.
[8] Slooter AJ, Bots ML, Havekes LM, et al. Apolipoprotein E and carotid artery atherosclerosis: the Rotterdam Study. Stroke 2001;32:1947–52.
[9] Haraki T, Takegoshi T, Kitoh C, et al. Carotid artery intima-media thickness and brachial artery flow-mediated vasodilation in asymptomatic Japanese male subjects amongst apolipoprotein E phenotypes. J Intern Med 2002;252:114–20.
[10] Volcik KA, Barkley RA, Hutchinson RG, et al. Apolipoprotein E polymorphisms predict low density lipoprotein cholesterol levels and carotid artery wall thickness but not incident coronary heart disease in 12,491 ARIC Study participants. Am J Epidemiol 2006;164:342–8.
[11] Beilby JP, Hunt CC, Palmer LJ, et al. Apolipoprotein E gene polymorphisms are associated with carotid plaque formation but not with intima-media wall thickening: results from the Perth Carotid Ultrasound Disease Assessment Study (CUDAS). Stroke 2003;34:869–74.
[12] Elosua R, Ordovas JM, Cupples LA, et al. Association of APOE genotype with carotid atherosclerosis in men and women: the Framingham Heart Study. J Lipid Res 2004;45:1868–75.
[13] Debette S, Lambert JC, Gariepy J, et al. New insight into the association of apolipoprotein E genetic variants with carotid plaques and intima-media thickness. Stroke 2006;37:2917–23.
[14] Kogawa K, Nishizawa Y, Hosoi M, et al. Effect of polymorphism of apolipoprotein E and angiotensin-converting enzyme genes on arterial wall thickness. Diabetes 1997;46:682–7.
[15] Sass C, Zannad F, Herbeth B, et al. Apolipoprotein E4, lipoprotein lipase C447 and angiotensin-I converting enzyme deletion alleles were not associated with increased wall thickness of carotid and femoral arteries in healthy subjects from the Stanislas cohort. Atherosclerosis 1998;140:89–95.
[16] Hanon O, Girerd X, Luong V, et al. Association between the apolipoprotein E polymorphism and arterial wall thickness in asymptomatic adults. J Hypertens 2000;18:431–6.
[17] Tabara Y, Kohara K, Nakura J, et al. Risk factor-gene interaction in carotid atherosclerosis: effect of gene polymorphisms of renin-angiotensin system. J Hum Genet 2001;46:278–84.
[18] Kweon SS, Shin MH, Jeong SK, et al. Cohort profile: the Namwon Study and the Dong-gu Study. Int J Epidemiol 2013. http://dx.doi.org/10.1093/ije/dys244.
[19] Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with Hhal. J Lipid Res 1990;31:545–8.
[20] Shin MH, Kim HN, Cui LH, et al. The effect of apolipoprotein E polymorphism on lipid levels in Korean adults. J Korean Med Sci 2005;20:361–6.
[21] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.

[22] Choi YH, Kim JH, Kim DK, et al. Distributions of ACE and APOE polymorphisms and their relations with dementia status in Korean centenarians. J Gerontol A Biol Sci Med Sci 2003;58:227–31.
[23] Chun S, Min WK, Kim JW, et al. Apolipoprotein E polymorphism and serum lipoprotein(a) concentrations in a Korean male population. Ann Clin Biochem 2001;38:129–34.
[24] Katsuya T, Baba S, Ishikawa K, et al. Epsilon 4 allele of apolipoprotein E gene associates with lower blood pressure in young Japanese subjects: the Suita Study. J Hypertens 2002;20:2017–21.
[25] Howard BV, Gidding SS, Liu K. Association of apolipoprotein E phenotype with plasma lipoproteins in African-American and white young adults. The CARDIA Study. Coronary Artery Risk Development in Young Adults. Am J Epidemiol 1998;148:859–68.
[26] Paternoster L, Martinez Gonzalez NA, Lewis S, et al. Association between apolipoprotein E genotype and carotid intima-media thickness may suggest a specific effect on large artery atherothrombotic stroke. Stroke 2008;39:48–54.
[27] Maezawa I, Nivison M, Montine KS, et al. Neurotoxicity from innate immune response is greatest with targeted replacement of E4 allele of apolipoprotein E gene and is mediated by microglial p38MAPK. FASEB J 2006;20:797–9.
[28] Roselaar SE, Daugherty A. Apolipoprotein E-deficient mice have impaired innate immune responses to *Listeria monocytogenes* in vivo. J Lipid Res 1998;39:1740–3.
[29] Lynch JR, Tang W, Wang H, et al. APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. J Biol Chem 2003;278:48529–33.
[30] Pepe MG, Curtiss LK. Apolipoprotein E is a biologically active constituent of the normal immunoregulatory lipoprotein, LDL-In. J Immunol 1986;136:3716–23.
[31] Jofre-Monseny L, Minihane AM, Rimbach G. Impact of apoE genotype on oxidative stress, inflammation and disease risk. Mol Nutr Food Res 2008;52:131–45.
[32] Bennet AM, Di Angelantonio E, Ye Z, et al. Association of apolipoprotein E genotypes with lipid levels and coronary risk. J Am Med Assoc 2007;298:1300–11.
[33] Schaefer EJ, Lamon-Fava S, Cohn SD, et al. Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. J Lipid Res 1994;35:779–92.
[34] Ophir G, Amariglio N, Jacob-Hirsch J, et al. Apolipoprotein E4 enhances brain inflammation by modulation of the NF-kappaB signaling cascade. Neurobiol Dis 2005;20:709–18.
[35] Paternoster L, Martinez-Gonzalez NA, Charleton R, et al. Genetic effects on carotid intima-media thickness: systematic assessment and meta-analyses of candidate gene polymorphisms studied in more than 5000 subjects. Circ Cardiovasc Genet 2010;3:15–21.