The Effect of Apolipoprotein E Polymorphism on Lipid Levels in Korean Adults

The aim of this study was to determine the effects of polymorphisms in the apolipoprotein E gene (*APOE*) on lipid levels in Korean adults and to investigate the interactions between these polymorphisms and environmental factors in determining lipid levels. We performed a cross-sectional study of 1,900 subjects (668 men and 1,232 women; 45-74 yr old) in Namwon, Korea, in 2004. *APOE* polymorphisms were determined by polymerase chain reaction and restriction enzyme analysis. Carriers of the *APOE*∗*E2* (*E2*) allele had significantly lower total cholesterol and low-density lipoprotein cholesterol (LDL-C) concentrations than did carriers of the *APOE*∗*E3* (*E3*) or *APOE*∗*E4* (*E4*) alleles, regardless of gender. The *APOE* allele type had significant effect on high-density lipoprotein cholesterol (HDL-C) and triglyceride levels in women, but not in men. The effect of *APOE* allele type on HDL-C levels was modified by age in women. In addition, in men, the effect of *APOE* allele type on triglyceride levels was modified by smoking. These findings highlight the important effect of gene-environment interactions on lipid levels.

Key Words : *Apolipoproteins E; Polymorphism, Genetic; Lipids; Gene-Environment Interaction*

Min-Ho Shin, Hee Nam Kim*,
Lian-Hua Cui†, Sun-Seog Kweon,
Kyeong-Soo Park, Heon Heo,
Hae-Sung Nam‡, Seul-Ki Jeong§,
Eun-Kyung Chung‖, Jin-Su Choi‖

Department of Preventive Medicine, Seonam University
College of Medicine, Namwon, *Genomic Research
Center of Hematopoietic Diseases, Chonnam National
University Hospital, Gwangju, Korea; Department of
Preventive Medicine†, Yanbian University Medical
College, Yanbian, China; Department of Preventive
Medicine‡, Chungnam University College of Medicine,
Daejeon; Department of Neurology§, ‖, Chonbuk
National University Hospital, Jeonju; ‡Department of
Preventive Medicine, Chonnam National University
College of Medicine, ‖Chonnam National University
Research Center of Medical Sciences, Gwangju, Korea

Address for correspondence
Eun-Kyung Chung, M.D.
Department of Preventive Medicine, Chonnam National
University College of Medicine, Chonnam National
University Research Center of Medical Sciences,
5 Hak 1-dong, Dong-gu, Gwangju 501-746, Korea
Tel : +82.62-220-4175, Fax : +82.62-233-0305
E-mail : sehyukmom@hanmail.net

INTRODUCTION

Apolipoprotein E (apoE) is a plasma protein that serves as a ligand for low density lipoprotein receptors and, through its interaction with these receptors, participates in the transport of cholesterol and other lipids among the various cells in the body (1). The *APOE* gene is located at chromosome 19q13.2 and has three common variants (*APOE*∗*E2*, *APOE*∗ *E3*, and *APOE*∗*E4*; *E2*, *E3*, and *E4*, respectively) and six different genotypes (*E2/E2*, *E2/E3*, *E2/E4*, *E3/E3*, *E3/E4*, and *E4/E4*) (2, 3). Allelic variation of APOE has been shown to influence the levels of serum lipids. Previous studies have consistently shown that total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) are highest in *E4* carriers, intermediate in *E3* carriers, and lowest in *E2* carriers (4, 5); however, some reports have shown no effect of *APOE* genotype on LDL-C levels (6, 7). In contrast, the associations between *APOE* genotype and triglyceride and high density lipo-

protein cholesterol (HDL-C) levels are less clear among different populations (5, 8). Moreover, the association between *APOE* genotype and lipid levels may be modulated by factors such as age, sex, ethnicity, diet, smoking behavior, alcohol consumption, and the presence of diabetes mellitus (3, 9, 10). The aim of this study was to evaluate the association between *APOE* polymorphisms and lipid levels in a Korean population, and to explore the gene-environment interactions in this population.

MATERIALS AND METHODS

Populations and study design

Potential participants for this study were recruited from residents of five administrative regions (three Myuns and two Dongs) of Namwon in the Jeonbuk Province of Korea. We

used national resident registration lists to identify potential participants. The study area contained an estimated 4,697 people (2,142 men and 2,555 women) who were between 45 to 74 yr old in 2004, the age limits for eligibility for the study. Among these 4,697 eligible subjects, 1,964 (41.8%) underwent a clinical examination following an interview. The participants included 694 men and 1,270 women, and the mean ($\pm$ standard deviation) ages of the men and women were 61.9 ($\pm$ 6.7) and 61.1 ($\pm$ 7.1) yr, respectively. The response rate of women (48.4%) was significantly higher than men (33.5%), but the difference of mean ages between the respondent and non-respondent was not significant in both sexes. Subjects with missing lipid measurements or lifestyle data, or with triglyceride levels $\geq$ 800 mg/dL (11) were excluded from the study. The *APOE* genotypes were categorized into three groups: *APOE*E2* (*E2/E2* and *E2/E3*), *APOE*E3* (*E3/E3*), and *APOE* *E4* (*E3/E4* and *E4/E4*). Subjects with the *E2/E4* genotype (4 men and 12 women) were excluded because of the expected opposing biological effects of the *E2* and *E4* alleles. Ultimately, 1,900 subjects (668 men and 1,232 women) formed the final study sample for the principal analysis. All participants provided informed consent, and the study was conducted in accordance with the approval of the institutional research ethics committee.

Anthropometric measurements

Anthropometric measurements of subjects wearing light clothing and no shoes were conducted by experienced research staff. Height was measured to the nearest 0.1 cm, and weight was measured in the upright position to the nearest 0.1 kg. The body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Waist circumference was measured to the nearest 0.1 cm at the end of a normal expiration by measuring from the narrowest point between the lower border of the rib cage and the iliac crest.

Lipid measurements

All the participants underwent at least 10 hr of overnight fasting before blood samples were obtained from an antecubital vein. Serum was separated on-site and was stored at -70℃ until analyzed. The concentrations of TC, HDL-C, and triglycerides were measured by an automatic analyzer (AU5400, Olympus, Japan), and LDL-C was estimated using the formula of Friedewald et al. (12). The fasting blood glucose level was measured using an enzymatic method.

Measurement of environmental factors

The questionnaire administered to all participants was designed to determine the history of type 2 diabetes mellitus and lifestyle factors such as alcohol consumption, smoking, and exercise habits. The subjects were categorized according to their smoking habits as current smokers, ex-smokers, and non-smokers. Type 2 diabetes mellitus was defined as a self-reported history (having been informed by a physician that diabetes was present) or as a fasting blood glucose level $\geq$ 126 mg/dL.

ApoE genotyping

Genomic DNA was extracted from peripheral blood with a AccuPrep Genomic DNA Extraction Kit (Bioneer, Seoul, Korea) according to the manufacturer's protocol. *APOE* genotypes were determined as described by Hixson and Vernier, with slight modification (13). A 244-bp fragment of the *APOE* gene that spans the two polymorphic sites at codons 112 and 158 was amplified by polymerase chain reaction in a DNA thermal cycler (PTC-200; MJ Research, Inc, Watertown, MA, U.S.A.) using the primers 5′-ACA GAATTCG-CCCCGGCCTGGTACAC-3′ and 5′-TAAGCTTGGCA-CGGCTGTCCAAGGA-3′. The PCR amplifications were carried out in 10 μL reaction volumes containing 0.25 mM each dNTP, 1.5 mM magnesium chloride, 0.75 U of f-*Taq* polymerase (Solgent, Seoul, Korea), 50 ng of genomic DNA, and 0.25 μM of each of the primers. The PCR cycling conditions were 94℃ for 5 min, followed by 40 cycles of 94℃ for 30 sec, 65℃ for 40 sec, and 72℃ for 90 sec. A final extension phase of 5 min at 72℃ was included at the end of the protocol. The PCR products were digested with 5 units of *Hha*I (TaKaRa Co. Ltd., Kyoto, Japan) and the fragments were separated by electrophoresis in 10% polyacrylamide gels. After electrophoresis, the gels were treated with ethidium bromide and the DNA fragments were visualized using ultraviolet illumination.

Statistical analysis

The differences between sexes were compared using Student's unpaired t-test for continuous data and the chi-square test for categorical data. Chi-square tests were conducted to compare the differences between the observed and expected frequencies, assuming Hardy-Weinberg equilibrium. Because the distributions of TC and HDL-C were skewed, the data were log-transformed prior to the analysis. Analysis of covariance (ANCOVA) was carried out using the general linear model procedure to exclude the effects of potential confounders (such as age, BMI, waist circumference, smoking, alcohol consumption, exercise, and diabetes mellitus) on lipid levels. The interaction effect between the APOE allele and demographic (sex and age) and environmental (smoking, alcohol consumption, exercise, and diabetes mellitus) factors was tested by introducing corresponding interaction terms in the ANCOVA model. Bonferroni tests were applied to correct for multiple comparisons. All analyses were performed using SPSS software version 11.0 (SPSS, Chicago, IL, U.S.A.).

RESULTS

The descriptive characteristics of the study subjects are presented in Table 1. The percentages of men who were current smokers, consumed alcohol, engaged in regular exercise, or

Table 1. Characteristics of the subjects (n=1,900) by gender

	Men (n=668)	Women (n=1,232)	p*
Age (yr)	62.0±6.7	61.2±7.1	0.026
Body mass index (kg/m²)	23.8±2.9	25.0±3.2	<0.001
Waist circumference (cm)	86.5±8.2	87.8±8.8	0.002
Current smoker (%)	35.9	3.7	<0.001
Alcohol consumption (%)	62.9	26.2	<0.001
Regular exercise (%)	23.2	14.9	<0.001
Diabetes mellitus (%)	11.2	7.2	0.003
Total cholesterol (mg/dL)	190.5±39.3	202.0±40.0	<0.001
HDL cholesterol (mg/dL)	46.0±13.0	47.2±12.3	0.050
LDL cholesterol (mg/dL)	109.1±35.7	123.4±36.1	<0.001
Triglycerides (mg/dL)	177.0±113.5	156.6±96.7	<0.001
Apolipoprotein E genotypes[†]			
E2/E2	0.3	0.6	
E2/E3	10.3	9.1	
E2/E4	0.6	1.0	0.177
E3/E3	75.3	72.9	
E3/E4	12.6	15.3	
E4/E4	0.7	0.9	

Unless otherwise indicated, data are given as mean±standard deviation. HDL, high density lipoprotein; LDL, low density lipoprotein.
*p value, determined by t-test or chi-square test as appropriate, [†]The sample consisted of 1,916 individuals (672 men, 1,244 women), including the 16 subjects with the APOE∗E2/E4 genotype (4 men, 12 women).

had diabetes mellitus were significantly higher than the corresponding percentages of women; however, women had significantly greater mean BMI and waist circumference figures than did men. The TC, LDL-C and HDL-C levels were significantly higher in women than in men, but the triglyceride levels were higher in men. The distribution of the APOE polymorphism in our study population was in Hardy-Weinberg equilibrium, and not significantly different in men and women. The genotype distribution was similar to those observed in other Korean studies (14, 15) .

The relationship between the APOE genotypes and the serum lipid concentrations for male and female subjects is presented in Table 2. The APOE allele type had a significant effect on TC and LDL-C levels in both men and women ($p<0.05$). E2 carriers had significantly lower TC and LDL-cholesterol concentrations than did E3 or E4 carriers. The APOE allele type had no significant effect on HDL-C levels in men, but there was a linear trend ($p=0.04$). Among women, E2 or E3 carriers had significantly higher HDL-C levels than did E4 carriers. The APOE allele type had no significant effect on triglyceride levels in men; however, among women, E4 carriers had the highest and E3 carriers had the lowest triglyceride levels. The associations between the APOE allele and lipid levels were not modified by sex (Table 2).

Table 3 shows the interaction effects between the APOE alleles and age and environmental factors in men and women. In women, the effect of the APOE allele type on HDL-C levels differed with age (Table 3). In women <60 yr old, E2 and E3 carriers had significantly higher HDL-C levels than did E4 carriers (Fig. 1). This pattern was similar to that in men

Table 2. Total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglyceride levels according to APOE allele

	E2 (E2/2, E2/3) (n=72 Male, 120 Female)	E3 (E3/3) (n=506 Male, 890 Female)	E4 (E4/4, E3/4) (n=90 Male, 222 Female)	p*	p trend[†]	Interaction[‡] (Sex × APOE allele)
Total cholesterol						
Male	179.8 (170.8-188.8)	191.5 (188.1-194.9)[§]	193.2 (185.1-201.2)	0.045	0.041	
Female	188.7 (181.9-195.9)	202.2 (199.7-204.8)[§]	208.1 (203.0-213.3)[§]	<0.001	<0.001	0.623
LDL cholesterol						
Male	92.9 (84.9-100.9)	110.5 (107.6-113.5)[§]	114.0 (106.8-121.1)[§]	< 0.001	<0.001	
Female	107.8 (101.4-114.1)	124.6 (122.6-126.9)[§]	127.3 (122.6-132.0)[§]	<0.001	<0.001	0.947
HDL cholesterol						
Male	46.0 (43.4-48.8)	44.5 (43.5-45.5)	42.4 (40.2-44.7)	0.117	0.040	
Female	46.3 (44.2-48.4)[¶]	46.3 (44.5-47.0)[¶]	43.0 (41.6-44.5)	0.001	0.002	0.590
Triglyceride						
Male	163.2 (143.9-185.0)	146.1 (139.3-153.1)	152.6 (136.3-170.7)	0.241	0.530	
Female	138.6 (126.5-151.8)	130.3 (126.0-134.8)	152.7 (142.8-163.3)[∥]	<0.001	0.009	0.093

The mean lipid values were adjusted for age, BMI, waist circumference, smoking, alcohol, exercise, and diabetes mellitus, with 95% confidence intervals given in parenthesis. Log-transformed triglyceride concentration data were used in the analysis and the mean obtained using the anti-log transformation. LDL, low density lipoprotein; HDL, high density lipoprotein.
*p value by ANCOVA for global comparison between APOE alleles after adjusting for age, BMI, waist circumference, smoking, alcohol, exercise, and diabetes mellitus; [†]p value by ANCOVA for APOE alleles after adjusting for age, BMI, waist circumference, smoking, alcohol, exercise, and diabetes mellitus; [‡]p value by ANCOVA for interaction term (Sex × APOE allele) after adjusting for age, BMI, waist circumference, smoking, alcohol, exercise, and diabetes mellitus; [§]Significantly different from E2, $p<0.05$ (Bonferroni adjustment for multiple comparisons); [∥]Significantly different from E3, $p<0.05$ (Bonferroni adjustment for multiple comparisons); [¶]Significantly different from E4, $p<0.05$ (Bonferroni adjustment for multiple comparisons).
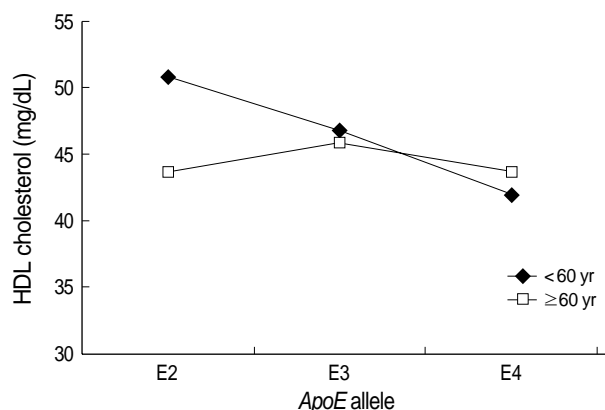
Fig. 1. Comparison of adjusted means of high density lipoprotein cholesterol (HDL-C) in females by *APOE* alleles and age subgroups. Mean HDL-C values are adjusted for age, BMI, waist circumference, smoking, alcohol, exercise, and diabetes mellitus. There was a significant interaction ($p<0.006$) between age group and *APOE* allele type on HDL-C levels. The *APOE* allele type had a significant effect on HDL-C levels in women <60 yr old ($p<0.001$) and in women ≥60 yr old ($p=0.049$).
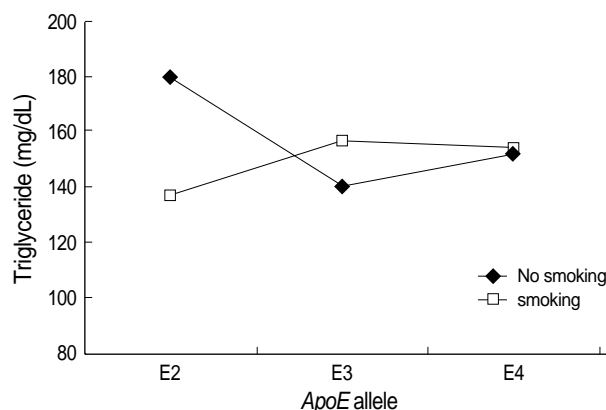

Fig. 2. Comparison of adjusted means of triglyceride levels in males by *APOE* alleles and smoking status. There was a significant effect of the interaction ($p=0.022$) between smoking status and *APOE* allele type on triglyceride levels. Mean triglyceride values are adjusted for age, BMI, waist circumference, alcohol, exercise, and diabetes mellitus. The *APOE* allele type had a significant effect on triglyceride levels in non-smokers ($p=0.018$), but no significant effect in smokers ($p=0.466$).

Table 3. *p*-values for the effects of interaction between *APOE* alleles and age and environmental factors on lipid levels

Interaction term*	Men (n=668)				Women (n=1,232)			
	TC	LDL-C	HDL-C	Triglyceride	TC	LDL-C	HDL-C	Triglyceride
APOE alleles+age	0.491	0.895	0.960	0.312	0.752	0.899	0.006	0.526
APOE alleles+smoking	0.521	0.676	0.402	0.022	0.671	0.601	0.089	0.418
APOE alleles+alcohol intake	0.476	0.893	0.680	0.592	0.496	0.635	0.727	0.342
APOE alleles+regular exercise	0.192	0.713	0.412	0.245	0.687	0.807	0.776	0.662
APOE alleles+diabetes mellitus	0.638	0.883	0.542	0.108	0.354	0.408	0.763	0.051

TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein.
p-value by ANCOVA for interaction term after adjusting for age, BMI, waist circumference, smoking, alcohol, exercise, and diabetes mellitus.

(Table 1). In women ≥60 yr old, the *APOE* allele type still had a significant effect on HDL-C levels ($p=0.049$); *E3* carriers tended to have higher HDL-C levels than *E2* or *E4* carriers, but there was no significant association of the *APOE* allele type with HDL-C levels after Bonferroni adjustment for multiple comparisons (result not shown). In addition, in men, the effect of *APOE* allele type on triglyceride levels was modified by smoking (Fig. 2). Among non-smokers, the triglyceride levels were highest in *E2* carriers and lowest in *E3* carriers. Among current smokers, *E3* carriers had the highest triglyceride levels and *E2* carriers had the lowest triglyceride levels, but these differences were not significant.

DISCUSSION

In this large population-based study, we have shown that *APOE* polymorphisms had a significant effect on lipid levels and that gene-environment interactions significantly affected lipid levels in a Korean population. We found that *E2* carriers had significantly lower TC and LDL-C levels than did *E3* or

E4 carriers. This finding is consistent with previous studies, in which TC and LDL-C levels were the highest in *E4* carriers, intermediate in *E3* carriers, and the lowest in *E2* carriers (5, 8, 16, 17). Our study also showed that *E4* carriers tended to have higher TC and LDL-C levels than *E3* carriers, although this difference was small and not significant. However, a meta-analysis (17) showed that subjects with *E3/E4* and *E4/E4* genotypes had significantly higher TC levels than did subjects with the *E3/E3* genotype. Frikke-Schmidt et al. (8) found that there was a stepwise increase in cholesterol and apoB levels, in both sexes, as a function of genotype (*E2/E2*, to *E2/ E3*, to *E2/E4*, to *E3/E3*, to *E3/E4*, to *E4/E4*) and alleles (*E2*, to *E3*, to *E4*). In a Singapore-based study (5), Tan et al. showed that *E4* carriers had higher TC and LDL-C levels than did *E3* carriers among ethnic Chinese, but that there were no significant allelotype-related differences among ethnic Malays or Asian Indians of either sex, with the exception of the LDL-C levels in ethnic Malaysian women. Therefore, differences in TC and LDL-C levels between *E3* carriers and *E4* carriers may be variable across populations.

Our study also showed that the *APOE* allele type had a no

significant effect on HDL-C levels in men, although a negative linear trend ($p=0.04$) was present; however, in women, E2 and E3 carriers had significantly higher HDL-C levels than did E4 carriers. Frikke-Schmidt et al. (8) showed that there was a stepwise decrease in HDL-C in women, but not in men, according to allelotype, as described above. In the Singapore study (5), Tan et al. showed that E2 carriers had the highest HDL-C levels and E4 carriers had the lowest HDL-C levels in the three ethnic groups studied. However, a meta-analysis (17) showed that there was no clear association between HDL-C levels and the APOE allele type, apart from the finding that HDL-C levels were significantly lower in E3/E4 carriers than in E3/E3 carriers.

We showed that female E4 carriers and male E2 carriers had the highest triglyceride levels, and that E3 carriers had the lowest triglyceride levels. This finding is in accordance with previous studies (5, 8, 14, 17, 18). In a study of Korean men, Chun et al. (14) showed that APOE allele type was associated with the median serum triglyceride levels in descending order from E2/E3, to E3/E4, to E3/E3. In the Singapore study (5), Tan et al. showed that E4 carriers had higher triglyceride levels, as compared with E2 and E3 carriers, with the exception of ethnic Malaysian males, in whom triglyceride levels were the highest in E2 carriers. A meta-analysis (17) showed that triglycerides were higher in E2 carriers and E4/E3 carriers than in E3/E3 carriers. Frikke-Schmidt et al. (8) showed that E2/E2 and E4/E4 carriers had the highest triglyceride levels, whereas E3/E3 carriers had the lowest triglyceride levels in both sexes. The higher levels of triglycerides observed in subjects carrying the E2 allele can be explained by their slower plasma clearance of chylomicron and VLDL remnants (17, 19, 20).

The association between APOE polymorphism and triglyceride and HDL-C levels are not entirely clear among different populations (4, 5, 8, 21). Gene-environment interaction may contribute to the discrepancies observed between studies. We found that the association between the APOE alleles and HDL-C levels was modified by age in women, and that the association between APOE alleles and triglyceride levels was modified by smoking status in men, and tended to be modified by the presence of diabetes mellitus in women ($p=0.051$). Previous studies have shown that HDL-C levels vary with physical activity, alcohol consumption, and diet (9, 22). Reznik et al. (23) showed that the association between APOE polymorphism and postprandial triglyceride clearance was modified by age, body weight, and fasting TG pool level. Some previous studies have also shown that the association between APOE polymorphism and lipid levels was modified by sex (24), but in our study, the associations between the APOE allele types and lipid levels were not modified by sex, with the exception of an association of borderline significance (0.093) for triglyceride levels. However, the underlying mechanisms responsible for the gene-environment interactions found in the present study are not clear. Such interactions

imply that, at the molecular level, there is synergy between gene products, or between gene products and the by-products of environmental insults, resulting in greater than additive effects (25).

The present study has some limitations. First, the relatively small sample size in men prevented us from showing statistically significant associations, although the patterns for TC and HDL-C levels that were observed in men were similar to those observed in women. Second, the proportion of the study population with risk factors such as smoking in women, diabetes mellitus, and the APOE*E2 or E4 alleles was low, and this limited the power of this study to detect gene-environment interactions.

In summary, we have shown that APOE polymorphisms had a significant effect on lipid levels in Koreans, that the association between the APOE allele type and HDL-C levels was modified by age in women, and that the association between the APOE allele type and triglyceride levels was modified by smoking status in men. These findings highlight the important effect of gene-environment interactions on lipid levels.

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