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Apolipoprotein E Polymorphism in Non-diabetic Patients with Acute Coronary Syndrome

In Jai Kim[1], Bum Kee Hong[1], Byoung Kwon Lee[1], Hyuck Moon Kwon[1], Dongsoo Kim[1], Eui Young Choi[1], Ki Hyun Byun[1], Jeong Ho Kim[2], Kyung Soon Song[2], and Hyun-Seung Kim[1]

Abstract

Since a decade ago, apolipoprotein (apo) E polymorphism has been focussed as a risk factor for cardiovascular disease. ApoE plays a central role as a receptor ligand for the uptake of lipoproteins from the circulation. There was an agreement on apoE polymorphism being one of the major risk factors for coronary artery disease (CAD) by its effects on lipid profiles. However, the effects of apoE have not been noted in all populations and conflicting results in the risk of CAD have been noted. Recently, in situ expression of apoE on the atherosclerotic plaque has been studied. We, therefore, investigated the effects of apoE genotype on patients with acute coronary syndrome, including unstable angina and acute myocardial infarction, in non-diabetic patients. While we could not find significant risk effects of apoE on coronary artery disease and lipid profiles on simple comparison with the normal control group, we could find significantly decreased frequencies of apo $\varepsilon 3$ allele in patients with acute coronary syndrome compared with stable angina patients (77.8% vs 88.8%). We suggest that the apoE genotype could be associated with acute coronary events in CAD and further study with in situ biochemical methods will be needed on the effects of apoE polymorphism on plaque stability.

Key Words: Apolipoprotein E, coronary artery disease, acute coronary syndrome

INTRODUCTION

Cardiovascular diseases, including coronary artery disease (CAD) and stroke, are the most important causes of mortality and morbidity in the world. Many studies have indicated that the classic cardiovascular risk factors, such as smoking, high blood pressure, and dyslipidemia are predictors of CAD events.[1]

ApoE plays a central role in the metabolism of cholesterol and triglycerides. Its main function is to serve as a receptor ligand for the uptake of lipoproteins from the circulation. In humans, apoE is a polymorphic protein with three different phenotypes, E2, E3, and E4, produced by three common alleles, $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$.[2]

From the most recent studies, there was an agreement on apoE polymorphism being a major atherosclerotic risk factor, especially the $\varepsilon 4$ allele. This has been explained by the effect of apoE on lipid profiles and by the direct effect of apoE genotypes. But, the effects of apoE may not be noted in the general population and controversy exists on the effects of apoE in patients with CAD.[3]

Recently, in situ expression of apoE on atherosclerotic plaques has been studied and the biologic role of apoE in situ investigated.[4-7] We investigated the influence of apoE polymorphism on clinical manifestations of coronary artery disease by focussing on the effect of apoE genotypes on acute coronary events.

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Departments of [1]Internal Medicine and [2]Clinical Pathology, Yonsei University College of Medicine, Seoul, Korea.
Address reprint request to Dr. B. K. Hong, Cardiology Division, Internal Medicine, Yongdong Severance Hospital, Yonsei University College of Medicine, Yongdong P.O. Box 1217, Seoul 135-720, Korea. Tel: 82-2-3497-3331, Fax: 82-2-573-0112, E-mail: bkhong@yumc.yonsei.ac.kr

MATERIALS AND METHODS

Subjects

Normal control group: 137 subjects were included in the normal control group. 40 subjects were people who visited Health Care Center at Yong-Dong Severance Hospital for a routine medical examination. They had no past or family medical history, and their clinical and laboratory data were normal. The other

97 subjects were patients who visited Yong-Dong Severance Hospital for atypical chest pain but were found to have normal coronary anatomy.

Patient group: 228 patients with significant CAD and typical chest pain were included in the patient group. Significant CAD was defined as luminal narrowing of $\geq 50\%$ of the reference vessel diameter on coronary angiography. Coronary angiography was performed at 1 to 7 days after the chest pain attack. Depending on the metabolic effects on the lipid profiles, the patient group was further divided into diabetic and non-diabetic subgroups. In the non-diabetic group, we classified subjects as patients with stable angina or patients with acute coronary syndrome, which includes unstable angina and acute myocardial infarction. Clinical diagnosis was made according to the definition of Braunwald,[8] with typical chest pain and history, cardiac enzymes, electrocardiogram, echocardiogram, and coronary angiography. With patients who had acute coronary syndrome, blood sampling for lipid profiles was acquired at stable state, 2 months after the coronary event, under a regular diet and without the usage of lipid lowering agents.

ApoE genotyping

Amplification of apoE sequences from genomic DNA for restriction isotyping: We used Hixon and Vernier's method in examining the apoE genotype.[9] Leukocyte DNA was amplified by PCR in a DNA Thermal Reactor (Hybaid, Tedington, Middlessex, UK) using oligonucleotide primers F4 (5'-ACAGAA TTCGCCCCGGCCTGGTACAC-3') and F6 (5'-TAA-GCTTGGCACGGCTGTCCA AGG-3'). We used the modified and simplified method. In addition to the buffer and nucleotide components described by the supplier of Taq polymerase, each amplification reaction contained 100 ng of Taq polymerase, 0.2 μM of each primer, 10% dimethyl sulfoxide, and 1.5 units of Taq polymerase in a final volume 50 μL.

Each reaction mixture was heated at 95°C for 5 minutes for denaturation, and subjected to 35 cycles of amplification by primer annealing (60°C for 30 sec.), extension (72°C for 40 sec.), and denaturation (95°C for 40 sec.) in a DNA Thermal Reactor. PCR products were 244 bp in size on electrophoresis with 1.5% agarose gel.

Restriction isotyping of amplified apoE sequences with HhaI and gel analysis: After PCR amplification, the PCR products were digested with the HhaI restriction enzyme for more than 3 hours at 37°C. Digested DNA were electrophoressed on 4% MetaPHore agarose (FMC Bioproducts, Rockland, ME, USA). DNA fragments were visualized by UV illumination.

Statistical analysis

The quantitative variables are presented as mean $\pm$ standard deviation (Mean $\pm$ S.D.) The statistical analysis was done with SPSS program (release 8.0 for windows, SPSS Inc, Chicago, IL, USA). The Chi-square test was done to compare the distribution of apoE genotype and the allelic frequency between the groups. To compare the means between each group, Mann-Whitney U test or ANOVA test was done. Criterion for statistical significance was $p < 0.05$.

RESULTS

There were six apoE genotypes (Fig. 1). The poly-

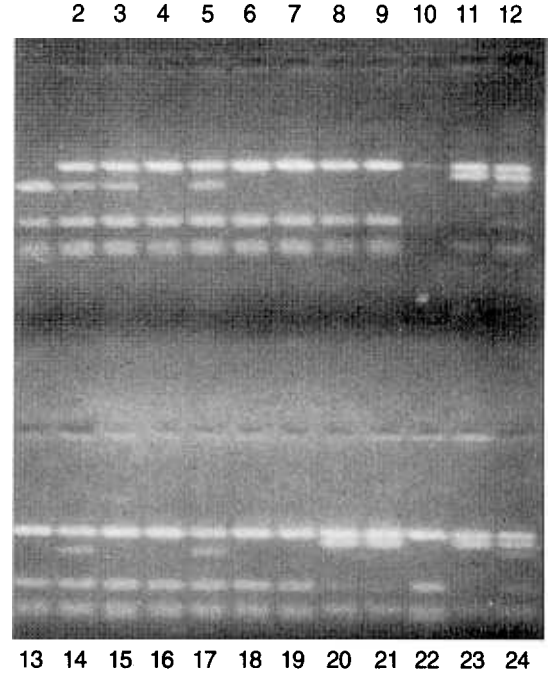

Fig. 1. ApoE Isotyping Pattern by Hha 1 fragment after PCR amplification. Lane 11, 23; E2/2, Lane 20, 21; E2/3, Lane 12, 24; E2/4, Lane 4, 6-10, 13, 15-16, 18-19, 22; E3/3, Lane 2-3, 5, 14, 17; E3/4, Lane 1; E4/4.

morphic gene frequency of apoE in the normal control group was 0.7% for ε2/2, 18.2% for ε2/3, 2.9% for ε2/4, 69.3% for ε3/3, 8.8% for ε3/4, and the allelic frequencies of apoE gene were 11.3% in ε2, 82.8% in ε3, and 5.8% in ε4. In patient with CAD, polymorphic gene frequencies of apoE were 0.9% for ε2/2, 14.9% for ε2/3, 0.4% for ε2/4, 66.7% for ε3/3, 16.7% for ε3/4, 0.4% for ε4/4 and the allelic frequencies of apoE gene were 8.6% in ε2, 82.5% in ε3, and 9.0% in ε4. There were no significant differences in polymorphic gene frequencies and allelic frequencies of apoE between the two groups (p=0.170) (Table 1). There were no significant clinical laboratory profiles between the diabetic and non-diabetic groups except higher triglyceride levels were detected in the diabetic CAD group (p=0.037) (Table 2). There were also no significant differences between the diabetic and non-diabetic CAD groups (p=0.877).

The allelic frequencies of apoE gene were 5.2% in

Table 1. Apolipoprotein E Genotype, Allelic Frequency between the Coronary Artery Disease Group and Normal Control Group

Apolipoprotein E		Coronary artery disease (n=228)	Normal (n=137)	χ^2	p value
Frequency by genotype (%)	ε2/2	2 (0.9)	1 (0.7)		
	ε2/3	34 (14.9)	25 (18.2)		
	ε2/4	1 (0.4)	4 (2.9)	9.055	0.107
	ε3/3	152 (66.7)	95 (69.3)		
	ε3/4	38 (16.7)	12 (8.8)		
	ε4/4	1 (0.4)	0 (0.0)		
Allelic frequency	ε2	0.086	0.113		
	ε3	0.825	0.828	3.542	0.170
	ε4	0.090	0.058		

Table 2. Apolipoprotein E Genotype, Allelic Frequency, Clinical and Laboratory Data between the Diabetic and Non-Diabetic Group in Patients with Coronary Arterial Disease

ApoE genotype and allele, clinical and laboratory data		Diabetic (n=73)	Non-diabetic (n=155)	χ^2	p value
Frequency by genotype (%)	ε2/2	0 (0.0)	2 (1.3)		
	ε2/3	12 (16.4)	22 (14.2)		
	ε2/4	0 (0.0)	1 (0.6)	2.058	0.841
	ε3/3	49 (67.1)	103 (66.5)		
	ε3/4	12 (16.4)	26 (16.8)		
	ε4/4	0 (0.0)	1 (0.6)		
Allelic frequency	ε2	0.082	0.090		
	ε3	0.836	0.816	0.263	0.877
	ε4	0.082	0.094		
Sex	Male : Female	45 : 28	107 : 48	1.219	0.170
Age	(Mean±S.D.)	60.18±7.87	59.35±9.51		0.604
Body mass index	(Mean±S.D.)	25.47±2.79	25.11±3.03		0.543
Total cholesterol	(Mean±S.D.)	200.85±37.29	202.05±40.34		0.958
Triglyceride	(Mean±S.D.)	189.77±57.03	170.03±50.05		0.037
LDL-cholesterol	(Mean±S.D.)	128.10±36.08	133.17±38.42		0.658
HDL-cholesterol	(Mean±S.D.)	34.79± 5.68	35.87±7.45		0.655
Lp (a)	(Mean±S.D.)	34.21±38.31	26.33±29.18		0.592

Table 3. Comparison of Apolipoprotein E Genotype, Allelic Frequency of the Angina Pectoris vs Myocardial Infarction Subgroups, and Stable Angina vs Acute Coronary Syndrome Subgroups in Patients with Non-Diabetic Coronary Arterial Disease

ApoE genotype and allele		Stable angina (n=58)	Acute coronary syndrome (n=97)
Frequency by genotype (%)	ε2/2	0 (0.0)	2 (2.1)
	ε2/3	5 (8.6)	17 (17.5)
	ε2/4	1 (1.7)	0 (0.0)
	ε3/3	46 (79.3)	57 (58.8)
	ε3/4	6 (10.3)	20 (20.6)
	ε4/4	0 (0.0)	1 (1.0)
		χ^2=10.084, p=0.073	
Allelic frequency	ε2	0.052	0.108
	ε3	0.888	0.778
	ε4	0.060	0.113
		χ^2=5.911, p=0.044	

ε2, 88.8% in ε3, and 6.0% in ε4 in patients with stable angina. In patient with acute coronary syndrome, the allelic frequencies of apoE gene were 10.8% in ε2, 77.8% in ε3, and 11.3% in ε4. There was a significant difference in allelic frequencies of apoE between the stable angina and acute coronary syndrome groups (p=0.044) (Table 3). Lipid profiles were not significantly different according to the polymorphic gene frequencies of apoE in the acute coronary syndrome subgroups of non-diabetic CAD patients.

DISCUSSION

The present study revealed that the ε3 allelic frequency was significantly lower, but ε2 and ε4 significantly higher, in patients with acute coronary syndrome than in patients with stable angina among non-diabetic CAD patients. Thus, we suggest that the apoE polymorphism might have a role in acute coronary events.

There are many differences in apoE allele frequencies between various populations due to ethnic origin.[3,10] Gerdes et al. reviewed 45 different population studies of ApoE polymorphism and grouped 8 different types of apoE allelic frequencies from these studies.[3] The results of the current study on apoE allele frequency in the normal population (Table 1) were similar with those of other korean studies.[11-13] In healthy individuals, between 5 and 15% of normal interindividual variation in plasma cholesterol levels can be attributed to common apoE polymorphism.[14] In many human populations, it was found that individuals with apoE2 display high levels of apoE and low levels of plasma cholesterol, LDL-cholesterol and apoB, whereas those with apoE4 show the opposite.[15] However, this has not been observed in all of the populations studied so far.[16-19] In the present study, there was a higher triglyceride level in the diabetic CAD group, thought to be from both the overproduction of VLDL in the liver and to a disposal defect in the periphery. However, we could not find any differences of lipid profiles in the CAD groups according to apoE polymorphism. There are still no reasonable explanations for the differences between populations, regarding the effect of apoE polymorphism on plasma cholesterol levels, although gender, ethnic origin, lifestyle and diet are often put forward as influencing factors.[19]

Besides the fact of apoE polymorphism having effects on lipid profiles, there are many cross-sectional and longitudinal studies demonstrating an association of allelic variations in the gene coding of apoE with the variation of risk for CAD.[20,21] The amount of interest on this subject is due to the fact between different population groups which have different mortality rates from coronary heart disease, there are many differences in the apoE allele frequencies. The differences of the allelic frequencies also exists within the Caucasian populations.[3,22] Thus, apoE polymorphism may contribute to inter-population variability in CAD risk and mortality rates. Several studies showed that disease onset, or disease severity of CAD

were influenced by apoE polymorphism.[23-25] Also, the regions with a high apoE4 frequency should present with an increased incidence of CAD.[26-28] However, conflicting results were shown in other studies including this study.[29] The allelic frequencies of the apoE gene in the CAD group were 8.6% in $\varepsilon 2$, 82.5% in $\varepsilon 3$, and 9.0% in $\varepsilon 4$, and there were no differences in polymorphic gene frequencies and allelic frequencies of apoE between the normal group and the patient group (Table 1). These conflicting results are explained by important differences in the selection criteria of hyperlipidemic individuals, considering ethnic origin, gender, behavioral habits and the method of diagnosing of CAD.[19]

Recent studies from angiographic, and pathologic studies have established a clear association between plaque fissuring or rupture and the development of the acute thrombotic coronary syndromes.[7,30] It has been known for many years that the defective expression of apoE, either absent expression or expression of variant forms, was associated with an increased risk of atherosclerotic vascular disease.[31] Abundant apoE is found in situ atherosclerotic vascular wall lesions and macrophages are the major source of apoE in these lesions.[4,5] ApoE expression may have direct impact on early lesion of atherogenesis and progression of atherosclerotic plaque.[32,33] It was also reported that in situ expressed ApoE regulates intracellular and extracellular cholesterol homeostasis of the vessel wall through contact of the novel ligand with circulating lipoproteins.[34,35] It suggested the importance of the local production of apoE in modifying intracellular and extracellular components of the plaque, such as lipid-laden foam cells, and in its rupture. Huang et al. suggested that a plasma fraction containing a gamma-migrating lipoprotein particle with apoE, from individuals expressing the apoE3/3 phenotype, stimulates cholesterol efflux 7- to 13-fold more from cultured fibroblasts compared to the same fraction of plasma from apoE2/2 or apoE4/4 individuals.[36] On the basis of these ideas, patients of the current study were subdivided into group of patients with acute coronary syndrome and patients with stable angina. In the present study, we could find a significant difference in allelic frequencies between stable angina and acute coronary syndrome subgroups of non-diabetic CAD patients (Table 3). The present study revealed that $\varepsilon 3$ allelic frequency was significantly lower in patients with acute coronary syndrome than in patients with stable angina among the non-diabetic CAD patients. However, the allelic frequency of both $\varepsilon 2$ and $\varepsilon 4$ were significantly higher in patients with acute coronary syndrome than in patients with stable angina among the non-diabetic CAD patients. The limitation of this study was that we did not investigate the direct effects of apoE polymorphism on plaque stability in vivo.

In conclusion, we suggest that apoE polymorphism could be a risk factor for acute coronary events and further study with in situ biochemical methods on plaque stability will be needed.

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