The Egyptian Journal of Medical Human Genetics (2016) 17, 99–103

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The Egyptian Journal of Medical Human Genetics

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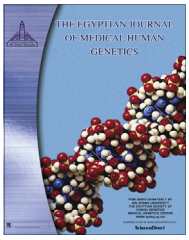

ORIGINAL ARTICLE

Apolipoprotein E gene polymorphism in Egyptian acute coronary syndrome patients

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Received 12 July 2015; accepted 17 August 2015
Available online 7 September 2015

KEYWORDS

Acute coronary syndrome;
Apolipoprotein E;
Genome association;
Sequence-specific-primer-PCR

Abstract *Background:* Apolipoprotein E (apo E) gene polymorphism was found to be associated with coronary artery disease in several studies.

In this investigation, we aimed to study the association between apo E gene polymorphism and acute coronary syndrome in Egyptian population.

Subjects and methods: The study included 200 patients with acute coronary syndrome (myocardial infarction and unstable angina), and 100 healthy controls. Anthropometric, clinical and lipid profile parameters were evaluated. Apo E genotyping was carried out using sequence-specific-primer (SSP)-PCR methodology.

Results: Є3/4 genotype frequency was higher in the patients than in the controls ($P < 0.05$), while Є2/3 genotype frequency was elevated in the controls than in the patients ($P < 0.05$). In addition, the frequency of Є4 isoform was higher in the patients compared to the controls ($P < 0.001$). Patients with Є3/4 and Є4/4 genotypes had significantly higher total cholesterol and low density lipoprotein cholesterol, and lower triglyceride levels than those with Є3/3 genotype. No significant differences in apo E genotype distribution were found between myocardial infarction and unstable angina patients.

Conclusion: Apo E gene polymorphism had a role in acute coronary syndrome, possibly through affecting plasma lipid parameters.

1. Introduction

Morbidities related to atherosclerosis, such as acute coronary syndromes (ACSs) including unstable angina and myocardial infarction are leading causes of mortality, and approximately seven million patients die annually due to coronary artery diseases [1]. Genetic factors were found to play a role in the pathogenesis of coronary artery disease [2]. Apolipoprotein E (apo E) is a 34-kDa protein found associated with several classes of plasma lipoproteins with a primary function in cholesterol and lipid transport [3]. Apo E is expressed in most cells of the body and the gene coding for apo E is located on the long arm of chromosome 19 (q13.32) [4]. Its gene contains

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Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2015.08.001

several polymorphic loci and two single-nucleotide polymorphisms (SNPs) at positions +2059 (T/C), located in the codon that codes for amino acid 112 and +2197 (C/T), that codes for amino acid 2196 of the apo E protein. Allelic variation within the Apo E gene has been shown to account for as much as 7% of the interindividual variability in low density lipoprotein cholesterol (LDL-C) and total cholesterol (TC). Multiple studies in human populations have demonstrated the associations of the Є4 isoform with increased LDL-C and of the Є2 isoform with decreased LDL-C levels [5]. The common isoforms of apo E are; Є2 (2059-T/2197-T), Є3 (2059-T/2197-C), and Є4 (2059-C/2059-C). The presence of T at position 2059 (2059-T) of the gene defines a cysteine at position 112 (Cys 112), whereas 2059-C defines an arginine for the same position (Arg 112). Similarly, 2197-C on the gene defines an arginine at position 158 (Arg 158), whereas 2197-T codes for cysteine for the same position (Cys 158) [6]. Apo E polymorphisms have been found to be associated with many lipid-related diseases and with circulating markers of inflammation like C-reactive protein [7–10].

The aim of the present investigation was to study the association of apo E gene polymorphism with acute coronary syndromes in Egyptian population.

2. Subjects and methods

2.1. Study subjects

The study included 200 patients with acute coronary syndromes (100 acute myocardial infarction patients and 100 unstable angina patients), enrolled from the coronary unit of Sohag University Hospital. 100 healthy subjects, matched for age and sex and resided in the same geographical region, with no previous history of coronary artery disease or atherosclerosis served as controls. Acute coronary syndromes (ACSs) have been defined as unstable angina or myocardial infarction with or without S-T segment elevation. Patients with unstable angina had ischemic chest pain within the preceding 48 hours with transiently depressed S-T segment and/or inverted T-wave. The diagnosis of myocardial infarction was done by the presence of two of the following: the electrocardiographic finding of S-T segment elevation of 1 mm in two or more successive leads, typical chest pain longer than 20 min' duration and an elevation in serial cardiac markers. Confirmation of the diagnosis by the presence of hypokinetic and akinetic segments at angiography [11]. The inclusion criteria for the patients were; no history of liver or renal disease and absence of non-atherogenic occlusion. The inclusion criteria for the controls were; no history of atherosclerosis, vascular disease, diabetes mellitus, renal or liver disease and normal ECG patterns. Written informed consents were obtained from all the enrollees and the study was carried out in accordance with the guidelines of the ethics committee of Sohag Faculty of Medicine.

2.2. Anthropometric parameters and blood pressure measurements

Body weight and height were measured in the morning while the participants were unclothed and without shoes. BMI was calculated as body weight (in kg) divided by height^2 (in m^2). Body mass index (BMI) was defined as the weight divided by the square of the height (kg/m^2). According to WHO, normal range BMI is from 18.50 to 24.99 kg/m^2, overweight $\geqslant$ 25 kg/m^2 and obese $\geqslant$ 30 kg/m^2 (WHO expert consultation, 2004) [12]. Blood pressure was read from the left arm while subjects remained seated. An average of 3 measurements was recorded. Hypertension was considered if the systolic BP $\geqslant$ 140 mmHg or the diastolic BP $\geqslant$ 90 mmHg or if the patient reported BP reducing medication use [13].

2.3. Blood collection and laboratory analysis

Approximately 5 ml venous blood samples were collected from the participants after an overnight fasting on EDTA tubes. The samples were centrifuged at 3000 rpm for 15 min and the buffy coat was used for DNA extraction using QIAamp kit supplied by Qiagen (USA). Plasma was used for the estimation of lipid parameters and blood glucose. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides (TG) and fasting glucose were determined using an enzymatic method on Cobas C 311 analyzer (Roche diagnostics, Germany). Low-density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald formula. The diagnosis of type 2 diabetes mellitus was done according to the American Diabetes Association Criteria [14], by presence of one or more of the following criteria: [1] treatment with hypoglycemic agents; [2] two diagnostic tests showing high blood glucose levels (fasting plasma glucose $\geqslant$ 126 mg/dL and/or 2-h plasma glucose $\geqslant$ 200 mg/dL during an oral glucose tolerance test).

2.4. Genotyping

We used an Apo E haplotype sequence-specific-primer (SSP)-PCR methodology that identifies in three PCR reactions the allelic haplotypes that determine the three main Apo E isoforms according to Pantelidis et al. [6]. Two forward and two reverse primers Table.1 were used for three primer mix reactions as follows; For Є2, the primer mix consists of primers 1 and 3, for Є3, the primer mix consists of primers 1 and 2, and for Є4, the primer mix consists of primers 2 and 4. For each reaction mixture, a pair of control primers was added, it amplifies two regions of chromosome 6 in the HLA-DR locus, to verify PCR amplification in the absence of haplotype specific amplification in each PCR reaction. The PCR cycling conditions were as follows: initial denaturation for 1 min at 96 °C;

Table 1 Primers used for SSP-PCR.

Primer	Sequence	Products
Primer-1 (forward)	5′-CGG ACA TGG AGG ACG TGT-3′	173 bp
Primer-2 (reverse)	5′-CTG GTA CAC TGC CAG GCG-3′	
Primer-3 (reverse)	5′-CTG GTA CAC TGC CAG GCA-3′	173 bp
Primer-4 (forward)	5′-CGG ACA TGG AGG ACG TGC-3′	
Control primers:		
Forward	5′-TGC CAA GTG GAG CAC CCAA-3′	785 and 1598 bp
Reverse	5′-GCA TCT TGC TCT GTG CAG AT-3′	

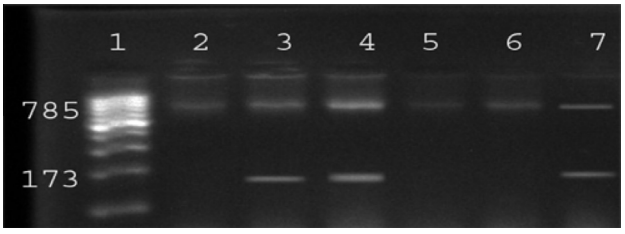

Figure 1a Lane 1; 100 bp ladder, lanes 2, 3 and 4 represent Є3/4 genotype and lanes 5, 6 and 7 represent Є4/4 genotype.

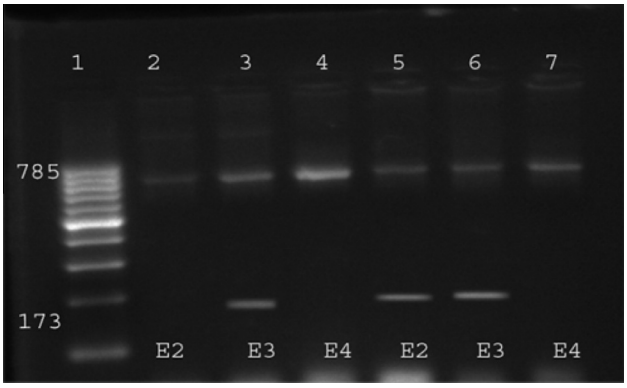

Figure 1b Lane 1; 100 bp ladder, lanes 2, 3 and 4 represent Є3/3genotype and lanes 5, 6 and 7 represent Є2/3 genotype.

Table 2 Demographic, clinical and laboratory data of the study groups.

Characteristic	Controls, n = 100	Patients, n = 200
Age (years)	54 ± 33	53 ± 5.07
BMI (kg/m^2)	25.3 ± 1.8	26.4 ± 3.2
Male gender, n (%)	35 (70)	140 (70)
Hypertension, n (%)	–	120 (60)
T2DM, n (%)	–	80 (40)
TC (mg/dL)	186.9 ± 12.4	208.6 ± 47.7***
HDL-C (mg/dL)	38.9 ± 3.5	32.3 ± 7.8***
TG (mg/dL)	109 ± 29.4	139.6 ± 29.3***
LDL-C (mg/dL)	101.8 ± 14.9	174 ± 66.9***

*** $P < 0.0001$.

followed by 5 cycles of 20 s at 96 °C, 45 s at 70 °C, and 25 s at 72 °C; 21 cycles of 25 s at 96 °C, 50 s at 65 °C, and 30 s at 72 °C; 4 cycles of 30 s at 96 °C, 60 s at 55 °C, and 120 s at 72 °C. The PCR products were analyzed by electrophoresis on 2% agarose gel stained with ethidium bromide. For all PCR reactions (Є2, Є3, and Є4), the presence of a 173 bp band indicated the presence of the specific Apo E haplotype. For the control primer pair, two products were obtained of 785 and 1598 bp (may be absent), Figs. 1a and b.

2.5. Statistical analysis

Data were expressed as mean ± SD or number and percent. Genotype distribution was tested for deviation from HWE (Hardy–Weinberg equilibrium) by x^2 analysis. Odd ratio with 95% confidence interval was calculated to compare the genotype frequencies in patients and controls. Continuous data were compared using Mann–Whitney and ANOVA tests and categorical data were compared using Chi-square test. A two-tailed value of $P < 0.05$ was considered statistically significant. All statistical calculations were performed using the computer program SPSS (Statistical Package for the Social Science; SPSS, Chicago, IL, version 16 for Microsoft Windows, USA).

3. Results

Our results showed no significant differences between the study groups regarding age, sex or BMI, while significant differences were found in plasma lipids and the presence of hypertension and T2DM, (Table 2). The genotype distribution was in accordance with HWE for both patients and controls. The most frequent genotype was Є3/3 (70% in the controls and 62% in the patients). Є3/4 genotype frequency was significantly elevated in the patients than in the controls ($P < 0.05$), while Є2/3 genotype frequency was significantly higher in the controls than in the patients ($P < 0.05$). Є4 isoform was found more frequently in patients compared to controls ($P < 0.001$), (Table 3). Patients with Є3/4 and Є4/4 genotypes had significantly higher TC and LDL-C and lower TG levels than those with Є3/3 genotype and patients with Є2/2, Є2/3, Є2/4 genotypes had lower LDL-C than Є3/3 genotype patients, (Table 4). No significant differences in apo E genotype distribution were found between myocardial infarction and unstable angina patients, (Table 4).

Table 3 Genotype and isoform distribution in the cases and controls.

Genotype	Controls, n = 100	Patients, n = 200	OR (95% CI)
Є2/2, n (%)	2 (2)	2 (1)	0.49 (0.07–3.6)
Є2/3, n (%)	20 (20)	21 (10.5)	0.47 (0.24–0.9)*
Є2/4, n (%)	1 (1)	7 (3.5)	3.5 (0.44–59.6)
Є3/3, n (%)	70 (70)	124 (62)	0.7 (0.4–1.17)
Є3/4, n (%)	6 (6)	42 (21)	4 (1.7–10.17)*
Є4/4, n (%)	1 (1)	4 (2)	2 (0.2–18.3)
Є2, n (%)	25 (12.5)	32 (8)	0.6 (0.35–1)
Є3, n (%)	166 (83)	311 (77.75)	0.7 (0.46–1)
Є4, n (%)	9 (4.5)	57 (14.25)	3.5 (1.7–7.3)**

* $P < 0.05$.
** $P < 0.001$.

Table 4 Clinical characteristics in acute coronary syndrome patients according to genotype.

	Є2/2, Є2/3, Є2/4, $n = 30$	Є3/3, $n = 124$	Є3/4, Є4/4, $n = 46$
BMI	25.3 ± 2.5	25.97 ± 2.6	27.2 ± 4*
Hypertension, *n* (%)	17 (57)	73 (59)	30 (65)
T2DM, *n* (%)	10 (33)	50 (40)	20 (43)
TC (mg/dL)	191 ± 19.5	191.6 ± 20.9	246.5 ± 66.6***
HDL-C (mg/dL)	34.4 ± 8	32.8 ± 7.9	31 ± 7.3
TG (mg/dL)	207.9 ± 61	196 ± 59	137.3 ± 31.5***
LDL-C (mg/dL)	132.8 ± 18.3	153.2 ± 52.5	188 ± 68.9***
Clinical presentation			
Unstable angina/MI	14/16	60/62	22/26

Patients were subdivided according to the isoforms (Є2, Є3 or Є4), due to the low frequency of Є2/2, Є2/4 and Є4/4 genotypes.
* $P < 0.05$.
*** $P < 0.0001$.

4. Discussion

Apo E gene polymorphism was found to be associated with variations in lipoprotein concentration and coronary artery disease [5]. In this study, Є3/3 genotype was the most frequent genotype found in the study population, a finding presented by other investigators [5,15]. Є3/4 genotype was more in the patients, while Є2/3 genotype was more frequent in the control group, in addition Є4 isoform frequency was significantly higher in the patients than in the controls. In accordance with our results, a meta-analysis by Xu et al. showed that MI patients had an increased frequency of Є4 and Є3/4 genotype, while Є2/3 genotype was more frequent in the controls [15]. However, a study of the association between apo E genotype and risk of coronary artery disease in White and African Americans showed no association between apo E alleles and coronary artery disease [5].

Our study showed that the Є2 isoform was associated with lower plasma levels of LDL-C and the Є4 isoform with higher levels, a result similar to those obtained in previous studies [5,10,16]. Regarding the elevated triglyceride levels in Є2 carriers, our results were similar to those obtained by other investigators [17–18]. The elevated triglyceride levels in Є2 carriers may be due to the impaired hepatic clearance of triglyceride rich lipoproteins as apo E2 is defective in binding to the LDL receptor [3].

The association between Є4 isoform and acute coronary syndrome can be explained based on the association between Є4 isoform and elevated plasma total cholesterol and LDL-C levels. Also, in a previous study Є4 isoform carriers were found to have high carotid artery intima-media thickness measures compared to other isoform carriers [5]. Elevated LDL-C levels are associated with a high risk of coronary artery disease and LDL oxidation could induce atherosclerosis [19].

Apo E plays an important role in the metabolism of cholesterol and triglycerides, by binding to its receptors mediating the clearance of chylomicron and remnant particles from plasma. In addition apo E was found to be associated with inflammatory markers and coagulation factors [20]. The three common isoforms; Є2, Є3 and Є4, have different receptor-binding abilities and could yield different circulating levels of cholesterol and triglycerides. Compared with Є3 homozygotes, carriers of the Є2 isoform have lower circulating cholesterol levels, whereas carriers of the Є4 isoform appear to have higher plasma levels of total and low-density lipoprotein cholesterol. According to the previous finding, we could explain the association between Є 4 isoform and acute coronary syndrome [15]. In conclusion, our study revealed an association between apo E gene polymorphism and acute coronary syndrome in Egyptian population, however, further studies are needed to elucidate the relation between apo E genotype, low density lipoprotein particle size and apo E protein levels.

Conflict of interest

The authors declare that there were no conflicts of interest.

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