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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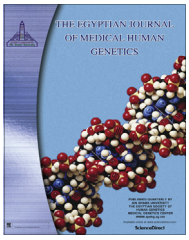

ORIGINAL ARTICLE

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′	