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Synergistic effect of ACE and AGT genes in coronary artery disease

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ABSTRACT

Polymorphisms of the renin-angiotensin system genes influence the pathogenesis of atherosclerosis and are connected with heart diseases. We explore the potential associations of ACE (I/D) and AGT (M235T) gene polymorphisms with coronary artery disease (CAD). A total of one hundred and twenty Egyptian patients (Sixty with CAD and sixty without CAD) and fifty healthy control subjects were included in the study. Genotyping of ACE (I/D) and AGT (M235T) were analyzed by the polymerase chain reaction (PCR) technique. Serum lipid profiles (total cholesterol, triglyceride, HDL-C) were measured by the enzymatic colorimetric method. Our data showed that the ACE D allele frequency ($P < 0.0001$; odds ratio [OR] = 2.538, 95% confidence interval [CI] = 1.468–4.388), DD genotype ($P < 0.0001$, OR = 0.239, 95% CI = 0.066–0.866), the frequency of AGT T allele ($P < 0.0001$, OR = 2.915, 95% CI = 1.666–5.097) and TT genotype ($P < 0001$, OR = 0.316, 95% CI = 0.078–1.280) were significantly more prevalent among CAD patients and may be predisposing to CAD. We further found that the concomitant presence of the ACE-DD and AGT-TT genotypes increased the risk of CAD ($P = 0.017$, OR = 0.120, 95% CI = 0.042–0.347). This study demonstrated the contribution of ACE (I/D) and AGT (M235T) gene polymorphisms individually or in combination to the presence of CAD risk in the Egyptian population. The ACE D allele and AGT T allele may be predictive in individuals at risk of developing CAD.

1. Introduction

Coronary artery disease (CAD) is a multifactorial disease affected by natural and hereditary elements. Family history of premature CAD, in addition to other hazard components, such as; smoking, obesity, diabetes, hypertension and dyslipidemia, are all interactive factors contributing to the occurrence of the disease (Rosendorff et al., 2015; El-Hussieny et al., 2015).

The renin – angiotensin system (RAS) assumes a critical part in the control of blood flow. Over action of this framework adds to the pathogenesis of an assortment of clinical conditions, including onset, movement, and result of atherosclerosis (Keith et al., 2007; Husain et al., 2015).

The angiotensin converting enzyme (ACE) is a gene situated on chromosome 17q23 that shows a 287-bp repeated *Alu* sequence insertion (I) or deletion (D) polymorphism in intron 16 (Pacurari et al., 2014). The homozygous DD genotype, which is connected with a two to triple increment in levels of ACE, may bring about an assortment of unfavorable cardiovascular impacts (Husain et al., 2015; Abdel-Aziz et al., 2012).

The angiotensinogen (AGT) gene, localized on chromosome 1q41-qter, encodes AGT; its length is 12 kb and consists of five exons interrupted by four introns as a single copy in the human genome (Kretowski et al., 2007). There is a single haplotype block at the AGT locus, and all regular single nucleotide polymorphisms recognized give off an impression of being in entire linkage disequilibrium with the most seriously considered M235T polymorphism (Kuo et al., 2008). In spite of the fact that the useful variation has not yet been completely distinguished, the T235 allele has been reliably connected with cardiovascular sickness and in a few reviews, yet not all with increased blood levels of AGT (Mehri et al., 2012).

The idea of genetic communication can be reached out to the presence of defensive and additionally suppressive hereditary variations, which when recognized can make an essential commitment to averting improvement of CAD or enhancing its clinical course (Musameh et al., 2015).

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Our purpose was to determine the potential associations of ACE (I/D) and AGT (M235T) gene polymorphisms, individually or in combination with the risk of CAD, and further, to find out their impact on the assessed biochemical parameters of the patients.

2. Patients and methods

2.1. Patients

A total of 120 Egyptian patients undergoing cardiac catheterization were enrolled in the study. They were selected from the Cardiology Department of The National Heart Institute, Giza, Egypt (from January 2013 to June 2013). Subjects were divided into two groups:

Group I): 60 patients (40 males and 20 females; their ages ranged from 32 to 69 years) with documented CAD. Documented CAD was diagnosed by:

a) Electrocardiograph tests, such as an electrocardiogram (ECG or EKG) or exercise stress tests to evaluate the electrical activity generated by the heart at rest and with activity.
b) Invasive techniques, such as cardiac catheterization, to get a closer look at the coronary arteries.

Group II): 60 participants (27 males and 33 females; their ages ranged from 27 to 72 years), they angiographically documented free CAD and suffered from CAD risk factors such as (diabetes, hypertension, obesity, smoking and dyslipidemia). They were considered as the patients without CAD or Non- CAD patients.

In addition, 50 healthy age and sex matched subjects (37 males and 13 females; their ages ranged from 25 to 55 years) were enrolled as a control group. They had no history of CAD, MI or stroke and did not suffer from any risk factors included in this study.

The study protocol was approved by The Medical Research Ethics Committee of The National research Centre (NRC) (Registration number -12-042). All subjects gave written informed consent to participate in this study.

2.2. Sampling

Peripheral venous blood samples (5 ml) were drawn under aseptic conditions from all subjects after an overnight fasting of 12 h. Samples were divided into two parts; one part (2 ml) of the whole blood was collected in EDTA coated tubes for DNA extraction for detection ACE and AGT gene polymorphisms. The second part (3 ml) of blood was allowed to clot for 10–15 min then centrifuged at 1000×g for 5 min for serum separation; the collected serum was stored at −20 °C in aliquots.

2.3. Biochemical analysis

Total cholesterol (TC), triglyceride (TG) and HDL-C were measured by an enzymatic-colorimetric method according to Allainet et al. (1974), Fossati and Prencipe (1982) and Burstein et al. (1980) respectively using the kits manufactured by STANBIO Laboratory, USA. LDL-C was calculated using the formula of Frieldewald et al. (1972).

2.4. DNA analysis

Genomic DNA was isolated from peripheral blood leukocytes using a spin column method according to the manufacturer's protocol (QIAamp Blood Kit; Qiagen GmbH, Hilden, Germany).

2.5. Genotyping of ACE (I/D) gene polymorphism

The 287-bp I/D polymorphism in intron 16 of the ACE gene; was detected by PCR using the following primers: forward 5′-CTGGA GACCACTCCCATCCTTTCT-3′ and reverse 5′-GATGTCGCCATCA CATTCGTCAGAT-3′ (Rigat et al., 1992). The reaction conditions were 35 cycles of 30 s at 94 °C, 30 s at 50 °C and 1 min at 72 °C followed by a final extension cycle of 72 °C for 7 min (T-Gradient Thermal Cycler, Biometra, Germany). The PCR products were analyzed by electrophoresis on 3% agarose gel then visualized under a UV transilluminator with 100-bp ladder. The amplification products were; 490 bp in of the I (insertion) allele and 190 bp of the D (deletion) allele (Fig. 1).

Due to the preferential amplification of the D allele, all the samples presenting the DD genotype were re-amplified using primers specific for insertion: forward 5′-TGGGACCACAGCGCCCGCCAC TAC-3′ and reverse 5′-TCGCCAGCCCTCCCATGCCCATAA-3′ (Shanmugam et al., 1993). The insertion-specific sequence was recognized with identical PCR condition except for annealing temperature at 67 °C. The seccond PCR reaction yielded a 335 bp DNA product in the presence of the I allele, and no product for homozygous DD samples (Fig. 2).

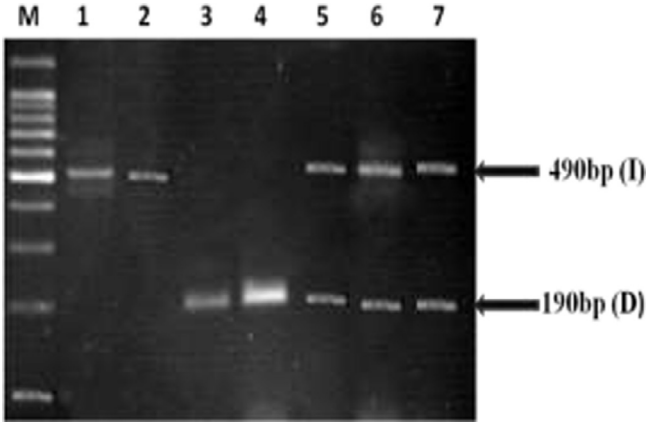

Fig. 1. Agarose gel electrophoresis of ACE (I/D) polymorphism, M lane: Molecular DNA ladder (100–1000 bp). Lanes 1 & 2: homozygous II genotype yielded 1 band of 490 bp. Lanes 3 & 4: homozygous DD genotype yielded 1 band of 190 bp. Lanes 5–7: heterozygous ID genotype yielded 2 bands of 490 bp and 190 bp.

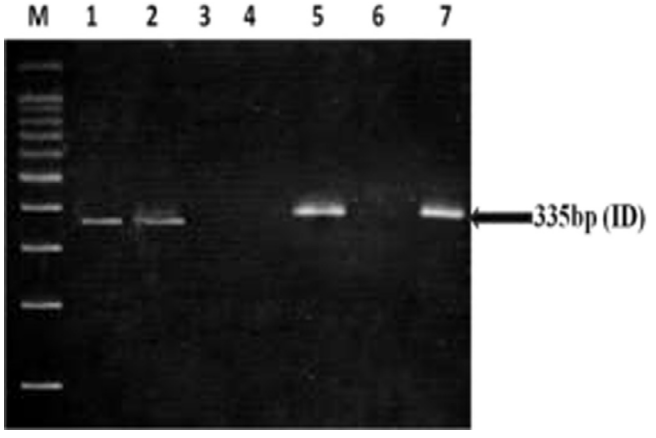

Fig. 2. Agarose gel electrophoresis of ACE Specific polymorphism. M lane: Molecular DNA ladder (100–1000 bp). Lanes 1, 2, 5 & 7: heterozygous ID genotype yielded 1 band of 335 bp. Lanes 3, 4 & 6: homozygous DD genotype showed no product.

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