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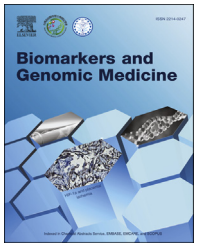

ORIGINAL ARTICLE

Angiotensin converting enzyme polymorphism in type 2 diabetes mellitus

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Abstract This study was undertaken to assess the frequency of angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism in patients with type 2 diabetes mellitus. A total of 162 patients with type 2 diabetes and 160 individuals without this disease were analyzed. From the diabetes group, 81 patients with cardiovascular risk (according to American Diabetes Association parameters) were selected to form another subgroup. For polymorphism identification, two polymerase chain reactions were performed: one reaction to identify all genotypes and a second one to confirm the presence of the I allele. The observed genotype frequencies were as follows: diabetes group I/I (19.1%), I/D (52.5%), D/D (28.4%); control group I/I (12.5%), I/D (55.6%), D/D (31.9%); and diabetes with cardiovascular risk group I/I (16.0%), I/D (59.3%), D/D (24.7%). No statistically significant difference was observed between the allelic and genotypic frequencies in the analyzed groups. Previous studies reported an association between the D allele and type 2 diabetes in Caucasian and East Asian populations. However, in mixed populations, such as those found in Brazil, such an association was not found. This fact does not discard the need for more studies on the frequencies of this polymorphism in the Brazilian population and the associations with risk factors, which can compromise the quality of life of diabetes patients.

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Introduction

Diabetes mellitus is a glucose homeostasis disturbance that affects 5% of the world population, and is characterized by absolute or relative insulin deficiency and/or resistance to this hormone. Type 2 diabetes mellitus (T2DM), considered the most frequent form of diabetes, is characterized by modifications in the insulin action and secretion, being generally associated with genetic susceptibility.[1] It is often associated with complications that include risk for developing atherosclerosis, cardiovascular diseases, nephropathy, retinopathy, and neuropathy, which makes this condition a serious public health problem.[1,2]

Angiotensin converting enzyme (ACE) catalyzes the conversion of angiotensin I to angiotensin II, which sustains and modulates blood pressure, volemic control, and regulation of kidney and systemic circulation, thereby influencing glomerular filtration, such as sodium tubular reabsorption. It also performs intracellular functions, including cellular hyperplasia and/or hypertrophy, with proinflammatory and pro-oxidant action, which results in cellular toxicity and apoptosis. Modifications in the renin—angiotensin—aldosterone system bring consequences to cells from endothelial and muscular tissues including inflammatory process, thrombotic events, and cardiovascular complications. Some reports demonstrated that a low degree of chronic inflammatory process predicts a risk of glucose intolerance and diabetes type 2.[3—11]

There are reports about several polymorphisms related to ACE, and one of these is the insertion/deletion (I/D) of the 287-bp fragment in the 16th intron of the ACE gene at the locus 17q23.3.[12] The enzyme encoded by the D allele presents higher activity than that encoded by the I allele.[12,13] Some studies associate the presence of the D allele and D/D genotype to predisposition to coronary artery disease, and myocardial infarction in diabetic and nondiabetic individuals.[14,15]

Considering the number of variables influencing the beginning, development, and prognosis of diabetes, it is therefore necessary to understand the relationship between the environmental and genetic factors related to this disease. Therefore, the aim of the present study was to evaluate the frequency of the ACE I/D polymorphism in diabetic and nondiabetic patients in the Brazilian population group.

Materials and methods

Participants

In this study, 162 peripheral blood samples from diabetic patients and 160 samples from individuals without this disease were analyzed. The diabetic patients group, nominated as the test group, was composed of participants aged between 37 and 89 years, representing both sexes and admixture ethnicity.[16] The diagnosis of diabetes was based on fasting blood sugar (reference value 70—99.00 mg/dL), HbA1c (4.8—6.0%), and evaluation of clinical history. The complementary tests were total cholesterol (<200 mg/dL), high-density lipoprotein cholesterol level (HDLc; >40 mg/dL), low-density lipoprotein cholesterol (LDLc; <130 mg/dL, Friedewald formula), and triglycerides (<150 mg/dL).

The American Diabetes Association[17] reported that diabetic patients with HDLc under 40 mg/dL, or LDLc above 100 mg/dL, or triglycerides above 150 mg/dL have higher risks of developing a heart disease.

Based on these standard data, 81 patients from the test group who had one or more cardiovascular disease risk factors cited previously were selected to form another study group named "With risk of cardiovascular disease". The control group was composed of healthy individuals aged between 40 and 85 years, representing both sexes and admixture ethnicity, who are voluntary blood donors.

DNA extraction and polymerase chain reaction of samples

After informed consent was obtained, 5 mL of peripheral blood sample was collected in tubes containing EDTA and stored at 8°C for up to 1 week. Genomic DNA extraction was performed using the modified phenol—chloroform method.[18] Polymerase chain reaction (PCR)[19] was performed in a volume of 25 μL that contained 1.5mM $MgCl_2$, 10× buffer without $MgCl_2$, 0.12mM dNTP, 0.8 U Taq Polymerase (Biotools, Madrid, Spain), 100 ng genomic DNA, and 0.2μM of each primer: 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ (sense) 5′-GAT GTG GCC ATC ACA TTG GTC AGA T-3′ (antisense). The cycling process was performed as follows: denaturation in 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 1 minute, and by the end an extension of 72°C for 5 minutes. In this first reaction, the primers that flank the polymorphic region allows the identification of a 490-bp fragment that corresponds to the insertion (I) and another fragment of 190-bp that corresponds to the deletion (D).

The possibility of 10% erroneous results has been described owing to a preferential amplification of the D allele in heterozygous genotypes. In order to correct this problem, a new PCR with specific primers for the I allele amplification was performed in all samples classified as D/D genotype. The primers used in this new reaction were 5′-TGG GAC CAC AGC GCC CGC CAC TAC-3′ (sense) and 5′-TCG CCA GCC CTC CCA TGC CCA TAA-3′ (antisense).[20] This second reaction was performed under the same conditions as the first one, except for the annealing temperature of 64°C. The presence of the I allele was assessed by the presence of a 335-bp fragment, and the absence of amplification indicated the presence of the D allele.

Statistical analysis

For the statistical analysis, the Chi-square test and Fisher's exact test were performed with an alpha of 5% for the polymorphism frequency using Statistica software, StatSoft Inc, Tulsa, OK, USA (version 7.0). As no available evidence favored any genetic models of inheritance for the polymorphism under study, the genotype frequencies were evaluated among each other, as follows: DD versus ID versus II and also DD versus ID + II (additive model), DD + ID versus II (dominant model) and DD versus ID + II (recessive model), in order to observe any possible codominant, additive,