Genetic Association of the ACE Gene Polymorphisms with Essential Hypertension in Korean Females

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Abstract: The renin-angiotensin system (RAS) genes have been extensively studied as etiologic essential hypertension (EH) candidate genes in human populations worldwide. The angiotensin I-converting enzyme (ACE) plays a key role in the RAS for the regulation of blood pressure. Recent studies on the association of ACE gene polymorphisms with EH and the related cardiovascular diseases presented conflicting results. Therefore, we investigated the association of three polymorphisms (I/D, G14480C and A22982G) in the ACE gene with EH in Koreans. We studied a sample population of 699 Koreans, comprising of 471 controls and 228 cases with EH, which were recruited from Cardiovascular Genome Center in Korea. The ACE gene polymorphisms were determined by polymerase chain reaction and SNP-IT assay. The allele frequency of G14480C polymorphism showed significant difference between normotensives and hypertensives in female group (P = 0.0353). After adjustment for age and BMI, logistic regression analysis indicated that the odds ratio (OR) of the ACE I carriers (II + ID) for a risk of EH over the DD genotype was 1.698 (95% CI, 1.006-2.864, P = 0.0458), and OR for the comparison of 14480 GG subjects versus GC and CC subjects combined was for EH 1.787 (95% CI, 1.076-2.967, P = 0.0236) in females. In haplotype analysis, major three haplotypes (ICA, IGG, and DGG) were significantly associated with EH in study populations. We conclude that ACE polymorphisms appeared to have apparent association with EH in Koreans, who have a more homogeneous genetic structure than any other ethnic groups.

Keywords: Angiotensin I-converting enzyme gene, essential hypertension, polymorphism, haplotype, Koreans

1. Introduction

The cause of essential hypertension (EH) is not clearly known, but it is assumed of multi-factorial disease due to the complex interaction between genetic and environmental factors. There are various genes may affect the onset mechanism of EH, but many studies focused on genes related to the renin-angiotensin system (RAS). RAS is composed of angiotensinogen (AGT), rennin, angiotensin converting enzyme (ACE), angiotensin II (AT2), angiotensin II type1 receptor (AGTR1), and angiotensin II type2 receptor (AGTR2). RAS controls blood pressure (BP), hydroelectrolyte metabolism and increase smooth muscle cell of blood vessel to play an important role in causing hypertension.

The ACE gene is located in the 17q23 chromosome of human and is composed of a 24,070 bp including 26 exons. ACE insertion/deletion (I/D) polymorphism is most widely used genetic marker in the studies evaluating the relationship of ACE gene polymorphism and cardiovascular disease (CVD) to the patients in a control group. ACE I/D polymorphism is shown as two allelic types, which has
insertion or deletion of 287 bp Alu repeat sequence in intron 16 of the ACE gene. Each type has one of II, ID, and DD genotype. Moreover, it is shown the highest activity with DD type and the lowest activity with II type, while ID was the modest in the study on the relationship between ACE genotype and serum ACE activity in Caucasians. It implied that ACE I/D polymorphism has relationship with BP control and hypertension. Studies on the relationship between ACE I/D polymorphism and EH has been pursued for various ethnic groups, but debate is still going on due to inconsistent result. O'Donnell et al. reported that Caucasian males had significant association between I/D polymorphism and EH, while the relation was not significant in African males. However, other studies showed that there is no significant association between I/D polymorphism and EH according to gender, while the results varied by age. These results indicated that the effect of I/D polymorphism on hypertension varied greatly by gender, age, and races. Recently there are active studies on the ACE polymorphism in Koreans, however, the results showed that ACE genotype distribution in Koreans is different from those of Caucasians, Oceanians, and Africans.

ACE G14480C polymorphism (rs4341) is a type that base guanine(G) at the intron 15 is replaced by cytosine(C), while A22982G(rs4363) has adenine(A) at the intron 25 is replaced by G. These two types of polymorphism occur at the edge of exon and intron, and they are reported to have close relationship with the increasing ACE activity. However, there are only few studies about the these polymorphism and there is no study for Koreans. It is more effective to study the relationship between gene and disease using haplotype which groups related opposite genotypes, in same chromosome or gene on different location, as a set, rather than comparing each of various polymorphism related to the disease. Although there are studied on the relation of ACE gene polymorphism of Korean with coronary artery disease(CAD), myocardial infarction(MI), and ischemic brain hemorrhage, there is no study on the relation of candidate gene and EH using haplotype analysis on relatively large case and control groups.

In the present study, we investigated the association study and haplotype analysis to elucidate the correlation between genetic variants of the ACE gene and EH in the Korean population.

2. Materials and Methods

2.1 Study Subjects
A total of 699 unrelated individuals (228 hypertensive cases and 471 normotensive controls) were enrolled from Cardiovascular Genome Center of Yonsei University College of Medicine. BP was measured with the use of a conventional mercury-column sphygmomanometer. Three recordings were taken on the right arm, each 5 min apart after participants had been sitting for more than 10 min of rest and mean values of measurements were used in this study. A hypertension patient was defined as patient with 140 mmHg and higher of systolic blood pressure(SBP) or 90 mmHg and higher of diastolic blood pressure(DBP) or who were being experienced any anti-hypertensive medication prior to measurement, but did not include patients with secondary hypertension, renal insufficiency, and diabetes. Control was chosen as healthy individuals with less than 140 mmHg of SBP and 90 mmHg of DBP without familial history of hypertension, diabetes, hepatic disease, and apparent CAD. Body mass index(BMI) was determined as an individual's body weight in kilograms divided by height in meters squared(kg/m²). We surveyed the age, gender, smoking, and disease history using the doctor's records and questionnaire of participants. The protocol was approved by the Ethics Committee of Yonsei University, and informed consent was obtained from all participants in this study.

2.2 Analysis on ACE Gene Polymorphisms
We collected 5 ml of total blood from the patient and control groups into ethylenediaminetetraacetic acid(EDTA) tubes for ACE gene polymorphism analysis. Genomic DNA was purified from whole blood using QIAamp® blood kit(Qiagen, Valencia, CA), according to the manufacturer's procedure. We measured the DNA concentration by spectrophotometer and aliquoted samples to 96-well or 48-well tubes by 25 ng/µl for the amplification purpose. We carried out polymerase chain reaction(PCR) using the following pair of primer to observe the insertion and deletion of Alu element with 287 bp length at the intron 16 of the ACE gene. The forward primer sequences are 5'-CTGGAGAGCCAC TCCCCATCTTCTTCT-3', and the reverse primer sequences are 5'-GATGTGGCCATCACATTGCAGA T-3', respectively. PCR reaction was performed in PCR thermal cycler(PTC-200, MJ Research, Waltham, MA) after mixing 25 ng of genomic DNA, 2.5 µM of each primer, 0.125 mM of dNTP, 50 mM KCl, 10 mM Tris-HCl(pH 8.3), 2.5 mM MgCl₂, 5% dimethyl sulfoxide(DMSO), and 1 unit of AmpliTaq polymerase (Applied Biosystems, Foster City, CA) to make it 25 µl. The