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Deletion polymorphism in the gene for angiotensin-converting enzyme is associated with essential hypertension in men born during the Pacific War

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Abstract

Age is a strong risk factor for hypertension in relation to vascular aging. Additional etiological factors include: lifestyle, genetic factors, and their interactions. The aim of this study is to examine whether an insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene is associated with essential hypertension in Korean born during the Pacific War. A total of 13,914 healthy subjects (8261 men, 5653 women) aged 20–79 years were examined. Subjects with abnormal renal, thyroid dysfunction, or electrolyte levels were excluded. Logistic regression analysis showed increased risk (OR = 1.15; 95% CI, 1.01–1.31) in men, but not in women (OR, 1.06; 95% CI, 0.89–1.26). However, after adjustment for age, obesity, cholesterol, alcohol consumption, and diabetes mellitus, increased risk in men was not significant (OR, 1.13; 95% CI, 0.98–1.42). Analyzed according to birth-year, DD genotype showed increased risk for hypertension in only a subgroup of men (adjusted OR, 1.56; 95% CI, 1.16–2.09; $p = 0.001$), born during the Pacific War (1941–1945 year). Findings suggest that the ACE DD genotype plays a role in the pathogenesis of essential hypertension, in conjunction with adverse environmental conditions in early life, with sex-related difference.

Keywords: Angiotensin; Gene; Hypertension; Blood pressure; Age; Sex; War

1. Introduction

Hypertension is a principal risk factor for myocardial infarction, stroke, and end-stage renal disease, with a high prevalence of 15–20% of the adult population in industrialized societies. Age is strongly linked to the development of essential hypertension of which etiologies include lifestyle and genetic factors (Lifton, 1996; Whelton, 1994). The renin–angiotensin system is involved in blood pressure regulation through its effect on vascular tone, renal hemodynamics, and sodium volume homeostasis (Laragh, 1992). Angiotensin-converting enzyme (ACE), an element of the renin–angiotensin system, catalyzes the conversion of angiotensin I to angiotensin II, a potent vasopressor, and inactivates bradykinin, a vasodilator. In humans, the insertion–deletion (I/D) polymorphism in intron 16 of the ACE gene is associated with serum ACE levels (Rigat et al., 1990). I/D polymorphism of the ACE gene has been implicated in the pathogenesis of cardiovascular diseases, including myocardial infarction (Cambien et al., 1992, 1994; Ludwig et al., 1995; Mattu et al., 1995; Nakai et al., 1994), although evidence for this hypothesis has varied in different studies (Lindpaintner et al., 1995; Winkelmann et al., 1996). In addition, common I/D polymorphism of the human ACE gene has been extensively investigated for a potential role in the etiology of hypertension in humans. The association of the ACE I/D polymorphism with differences in blood pressure has been observed in some (Duru et al., 1994; Morise et al., 1994; Barley et al., 1996; Zee et al., 1992; Nakano et al., 1998), but not other (Harrap et al., 1993; Schmidt et al., 1993; Vassilikioti et al., 1996; Kiema et al.,

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1996; Borecki et al., 1997) studies of hypertension. Two population-based studies reported a linkage between the ACE locus and hypertension (O'Donnell et al., 1998; Fornage et al., 1998). In Japan, a cohort study showed a significant association with hypertension and blood pressure (Higaki et al., 2000), but the other cross-sectional study did not find significant difference in blood pressure and prevalence of hypertension (Matsubara et al., 2002). Such discrepant results in previous studies may result from selection bias and limited sample size (Grimm and Rettig, 2002), which failed to control the effect of multiple environmental factors that influence blood pressure.

Conventionally, environmental factors that influence blood pressures include obesity, alcohol consumption, lack of physical activity, and high salt intake, which are related to lifestyle in adulthood (Whelton, 1994). Interestingly, intrauterine conditions have been recognized to be associated with adult hypertension (Barker et al., 1990), with suggestion of intrauterine programming in response to maternal malnutrition. South Korea had a hard experience of the Pacific War, in which most of the Korean people had been subjected to famine conditions and extreme distress. Such a historical background can provide an opportunity to test the combining effect of common genetic polymorphism and environmental factors. Although the etiology of hypertension is multifactorial, there has been no report combining the ACE genetic polymorphism with environmental factor.

The aim of present study, therefore, is to examine whether the ACE gene I/D polymorphism is associated with blood pressure and essential hypertension in a population, born during the Pacific War, using a large sample of 13,914 healthy Koreans aged 20–79 years.

2. Materials and methods

2.1. Study subjects

Apparently healthy men and women who volunteered in the preventive health care program, Samsung Seoul Hospital (Seoul, Korea), from January 1996 through February 1998 were examined. Over the 26 months, a total of 14,679 subjects between ages 20 and 79 years underwent health examination including ACE genotype determination. Subjects with thyroid dysfunction (serum thyroxin $\geq$ 154.4 n-nmol/L or thyroid stimulating hormone $\geq$ 5.5 mIU/L), renal dysfunction (serum creatinine $\geq$ 132.5 μmol/L), and low potassium level (<3.4 mmol/L) without diuretics use were excluded, because they may have had secondary hypertension or blood pressure change. Based on the criteria, 396 male and 369 female subjects were excluded from the study. A total of 13,914 subjects were selected. The study was approved by Institutional Review Board of Samsung Seoul Hospital. Informed consent was obtained from all the participants.

2.2. Measurement of laboratory examination and blood pressure

All participants were cautioned against alcohol consumption at least 72 h before examination. Blood was drawn at the fasting state, and handled together in a double-blind manner through analysis. Questionnaires were given to assess social and medical histories, obtained through self-administration, and additionally completed through interviews by physician or nurses. Alcohol consumption was categorized in glasses or bottles of Soju (Korean liquor, to be approximately 14 g per glass or 70 g per bottle) and the frequency (none to seven times per week/day). The multiplied values by amount were converted to an equivalent daily-consumed alcohol (ethanol gram).

The definition of hypertension was a current use of antihypertensive medication or blood pressures of systolic $\geq$140 mmHg and/or diastolic $\geq$90 mmHg. Blood pressure values were the mean value of two measurements (at least 3 min apart), using validated electronic sphygmomanometer with periodic calibration. Blood pressures exceeding 140/90 mmHg were repeatedly measured, following a pause of 30 min–3 h, and then the lowest values were input.

2.3. Determination of the ACE genotype

Genomic DNA was extracted from peripheral blood and amplified as previously described using the PCR with primers flanking the polymorphic region (Rigat et al., 1992). PCR products of the two alleles containing 490 and 190 base pairs were separated by electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining.

2.4. Statistical analyses

Continuous variables were compared among ACE genotypes using ANOVA with Duncan's method, and between group with and without disease using *t*-tests. χ^2 tests were used for categorical variables. Adjusted means of blood pressure, controlling for covariates, were compared using analysis of covariance (ANCOVA). Multiple regression analysis was done with covariates of age, body mass index, alcohol consumption, diabetes, and anti-hypertensive medication.

Under the assumption of a recessive effect of the D allele (DD compared with DI and II), odds ratio (OR) for hypertension and the 95% confidence interval (CI) were estimated by multiple logistic regression analysis. Body mass index (BMI) was calculated as the ratio bodyweight (kg) and squared height (m^2). Diabetes was considered in current user of oral hypoglycemic agents or insulin, or subjects with fasting glucose $\geq$7.0 mmol/L. BMI (27.0 kg/m^2), serum total cholesterol (6.2 mmol/L), diabetes mellitus, smoking (10 pack-year), and alcohol consumption (126 g/week) (Arkwright et al., 1982) were fitted as independent dichotomous variables. All analyses were gender-specific.