

## Alpha-Adducin Gly460Trp Polymorphism and Essential Hypertension in Korea

Previous studies have suggested that the Gly460Trp polymorphism of the alpha-adducin gene (*ADD-1*) is associated with salt sensitivity and primary hypertension. The results of linkage or association studies of *ADD-1* of different populations are controversial. This study investigated the relationship between the Gly460Trp polymorphism of *ADD-1* and essential hypertension in a Korean population. The subjects (n=903) were participants in a population-based study in Jangseong County, Korea. The Gly460Trp polymorphism of *ADD-1* was determined using a polymerase chain reaction method. The frequency of the 460Trp allele was 59.4% in normotensives and 61.1% in hypertensives ( $p=0.523$ ). The frequencies of the genotypes did not differ significantly between the hypertensive and normotensive groups (16.3% Gly/Gly, 45.8% Gly/Trp, and 38.0% Trp/Trp in normotensives; 16.2% Gly/Gly, 45.8% Gly/Trp, and 38.0% Trp/Trp in hypertensives;  $p=0.928$ ). After adjusting for other risk factors, Gly/Trp and Trp/Trp were not associated with hypertension (OR 1.00, 95% CI 0.65-1.53, Gly/Trp vs. Gly/Gly; OR 1.22, 95% CI 0.79-1.90, Trp/Trp vs. Gly/Gly). These findings suggest that the Gly460Trp polymorphism of *ADD-1* is not associated with hypertension.

Key Words : Hypertension; Adducin; Polymorphism, Single Nucleotide

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Received : 11 June 2004

Accepted : 22 July 2004

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\*This study was supported financially by Chonnam National University in the program, 2000.

## INTRODUCTION

Essential hypertension is currently seen as a multifactorial disease caused by multiple susceptibility genes, which are modulated by various environmental factors. Alpha-adducin (*ADD-1*) is a ubiquitously expressed cytoskeletal protein that appears to be involved in cell-to-cell contact (1), cell membrane ion transport (2), and signal transduction (3). Abnormalities of membrane sodium transport in the kidney play an important role in hypertension. Cusi et al. (4) reported that the Gly460Trp polymorphism of *ADD-1* was associated with a salt-sensitive form of hypertension. However, subsequent study results have been inconsistent (5). There is little information on its relation to hypertension in the Korean population. Therefore, we examined whether the Gly460Trp polymorphism of *ADD-1* was associated with hypertension in a Korean population.

## MATERIALS AND METHODS

### Subjects

The study subjects were 903 individuals who participat-

ed in a population-based study in Jangseong County, Korea, in August 2000. Informed consent for participation was obtained from all subjects. The participants completed a standard questionnaire on demographic characteristics, cigarette smoking, and alcohol consumption. Body mass index (BMI, kg/m<sup>2</sup>) was calculated from height and weight measurements. Blood pressures were measured using a common protocol with a Dinamap automated blood pressure reading device (Johnson & Johnson, New Jersey, U.S.A.). Two separate readings were taken. Our analysis is based on the average systolic blood pressure (SBP) and diastolic blood pressure (DBP). Hypertension was considered present if the average of two blood pressure measurements exceeded 140 mmHg systolic or 90 mmHg diastolic, or if the subjects were currently on antihypertensive medication.

### Genotyping

Genomic DNA was extracted from peripheral blood using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Seoul, Korea) according to the manufacturer's protocol. The Gly460Trp polymorphism of *ADD-1* was detected using a mutagenically separated polymerase chain reaction (PCR) (6, 7). Briefly, two allele-specific primers of different lengths (FP-

614G: 5' GGGGCGACGAAGCTTCCGAGGTAG-3' and FP-614T: 5' GCTGAACTCTGGCCCAGGCGACGAAGCTTCCGAGGATT-3') and a nonselective complementary strand primer (RP-614, 5' CCTCCGAAGCCCCAGCTAC-CCA-3') were used in a single-tube reaction assay. We confirmed the validity of the method for several samples using direct DNA sequencing. Five microliters of product was loaded per well onto a 10% polyacrylamide gel (19:1) using a microtiter array diagonal gel electrophoresis system (MADGE, MadgeBio, Grantham & Southampton, U.K.) and electrophoresed at 100 V for 100 min in 1 × Tris-borate-EDTA (TBE). The MADGE system uses a horizontal polyacrylamide gel to separate the MS-PCR product in a 96-well format (8). The PCR products were 220 and 234 bp for the 460Gly and 460Trp alleles, respectively

### Statistical analysis

The between-group demographic and blood pressure data were compared using Student's unpaired t-test for continuous data and the  $\chi^2$  test for categorical data. Allele frequencies were estimated by gene counting. Agreement between the genotype frequencies and Hardy-Weinberg equilibrium expectations was tested using the  $\chi^2$  test. To adjust for the contribution of confounding factors, we performed a multiple logistic regression. Statistical analysis was performed using Stata 8.0 (Stata Corp., College Station, Texas, U.S.A.).

**Table 1.** Characteristics of normotensive and hypertensive groups

	Normotensives (n=582)	Hypertensives (n=321)	p value
Male (%)	36.8	37.1	0.916
Age (yr)	55.9 ± 13.7	62.8 ± 11.1	<0.001
Height (cm)	157.1 ± 8.8	155.6 ± 9.1	0.004
Weight (kg)	56.4 ± 9.8	58.8 ± 10.3	0.001
BMI (kg/m <sup>2</sup> )	22.8 ± 3.1	24.3 ± 3.4	<0.001
SBP (mm Hg)	117.7 ± 11.8	152.2 ± 16.2	<0.001
DBP (mm Hg)	70.1 ± 7.7	85.8 ± 9.6	<0.001
Smoking (%)	35.7	38.6	0.389
Alcohol intake (%)	43.8	43.9	0.974
History of DM (%)	4.6	6.5	0.222

Values shown are the mean ± SD. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus.

**Table 2.** Association between alpha-adducin Gly460Trp polymorphism and hypertension

	Normotensives	Hypertensives	p value	Crude odds ratio (95% confidence interval)	Adjusted odds ratio* (95% confidence interval)
Alles					
Gly	473 (40.6)	251 (39.9)	0.523	1.00	1.00
Trp	691 (59.4)	391 (61.1)		1.07 (0.88-1.30)	1.13 (0.91-1.40)
Genotypes					
Gly/Gly	95 (16.3%)	52 (16.2%)	0.928	1.00	1.00
Gly/Trp	283 (48.6%)	147 (45.8%)		0.95 (0.64-1.41)	1.00 (0.65-1.53)
Trp/Trp	204 (35.1%)	122 (38.0%)		1.09 (0.73-1.64)	1.22 (0.79-1.90)

\*Adjusted by age, sex, body mass index, alcohol intake, smoking, and history of diabetes mellitus.

## RESULTS

The genotype distribution did not deviate significantly from Hardy-Weinberg equilibrium expectations (Gly/Gly, 16.3%; Gly/Trp, 47.6%; Trp/Trp, 36.1%;  $p=0.79$ ). Table 1 presents the baseline characteristics of all the subjects. Age, weight, height, body mass index, SBP, and DBP differed significantly between the hypertensive and normotensive groups. Sex, smoking habit, alcohol intake, and history of diabetes mellitus did not differ between the two groups. The frequency of the 460Trp allele was 59.4% in normotensives and 61.1% in hypertensives ( $p=0.523$ ). The genotype frequency did not differ significantly between the hypertensive and normotensive subjects ( $p=0.928$ ). After adjusting for age, sex, body mass index, smoking, alcohol intake, and history of diabetes mellitus, the odds ratio for hypertension associated with the presence of the Gly/Trp and Trp/Trp genotypes compared with the Gly/Gly were 1.00 (0.65-1.53) and 1.22 (0.79-1.90), respectively (Table 2).

## DISCUSSION

In this cross-sectional study, we investigated the association between an alpha-adducin gene polymorphism and essential hypertension in a Korean population. We found that the Gly460Trp polymorphism of *ADD-1* is not associated with essential hypertension. To our knowledge, this is the first report examining the relationship between *ADD-1* Gly460Trp gene polymorphism and hypertension in a sample from a general Korean population.

The frequency of the 460Trp allele varies in different populations. In this study, the frequency of the 460Trp allele was 59.4% in normotensives and 61.1% in hypertensives. These frequencies are very similar to those in Japanese (52-66%) (7, 9-11) and Chinese (42-56%) populations (12, 13). However, the frequency of the 460Trp allele is low in white populations (18% in Italy (4), 20% in France (4), and 27% in Scotland (14)) and very low (6%) in black South Africans (15). It is generally accepted that the frequency of a given variant allele in a population is determined by the biological fitness it confers in the presence of a given environment

(15). To achieve the functional characteristics needed to cope with an adverse environment, populations pick up the appropriate allele from the available genetic pool (5).

In this study, we could not detect any association between the Gly460Trp gene polymorphism and blood pressure. Cusi et al. (4) suggested that alpha-adducin was associated with a salt-sensitive form of hypertension. Subsequent studies have reported inconsistent results (5). There are a number of possible explanations for this inconsistency. The major consideration may be ethnic differences. In Asian studies, positive associations between the Gly460Trp gene polymorphism of *ADD-1* and blood pressure were found in three (10, 11, 16) of five studies (7, 9-11, 16) conducted in Japanese populations and in two (12, 13) of four (12, 13, 17, 18) in Chinese populations.

The mechanism by which *ADD-1* increases blood pressure is not known. *ADD-1* is thought to regulate ion transport via changes in the actin cytoskeleton (19). Adducin is thought to stimulate  $\text{Na}^+/\text{K}^+$ -ATPase, promoting sodium re-absorption by renal tubular cells (20). Trp/Trp individuals with this alteration in renal sodium handling will have increased sensitivity of blood pressure to sodium intake and are at increased risk for developing low-renin hypertension (21).

In summary, we failed to reveal a significant association between the Gly460Trp gene polymorphism of *ADD-1* and hypertension in a Korean population.

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