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CACNA1A Gene Polymorphism is Associated with Hypertension in Korean Population

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High blood pressure (BP) is the most frequent risk factor among metabolic syndrome components. The control of hypertension is very important to prevent the cardiovascular risk in metabolic syndrome. The dysfunction of calcium channel is responsible in the regulation of the vascular muscle contribution to hypertension. Calcium channel, voltage-dependent, P/Q type, alpha-1A subunit (*CACNA1A*) gene is located in brain and known to control the intracranial hypertension. In this study, we investigate whether the polymorphisms of *CACNA1A* gene is associated with hypertension. The 49 *CACNA1A* genotypes were determined using the Affymetrix Genotyping chip array in 92 hypertension and 279 control individuals from a Korean population. Logistic and multiple regression models were employed to analyze the genetic contributions of polymorphisms. Out of 49 polymorphisms, six SNPs (rs12611029, rs16035, rs7259944, rs10419472, rs17777900, and rs4926294) showed a significant association with hypertension in three alternative models (codominant, dominant, and recessive models; $P<0.05$ after adjusting for age and sex). Our results suggest that the *CACNA1A* gene may be associated with hypertension in the Korean population.

Key Words: CACNA1A, Calcium channel, Polymorphism, Hypertension, Metabolic syndrome

INTRODUCTION

Metabolic syndrome (MS) is a cluster of multiple cardiovascular risk factors. It is defined as the presence of two out of four conditions that result from insulin resistance: abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, and elevated plasma glucose. High blood pressure (BP) was believed to be the most frequent risk factor among metabolic syndrome components. The control of high normal or mild hypertension (clinical definition: BP > or = 130/ > or = 85 mmHg) is very important to prevent the cardiovascular risk in metabolic syndrome (Shimamoto, 2008). From the studies performed by many investigators, it is reasonable to conclude that calcium channel malfunction plays an important role in the vascular muscle contribution to hypertension (Hermsmeyer, 1993).

Voltage-gated Ca^{2+} channels have been functionally differentiated according to their inactivation properties into either transient (T-type) or long lasting (L-type) currents. Accordingly, N (neuronal), P (Purkinje cell), Q (granular cell) and R (toxin-resistant) channels can be distinguished depending on their tissue expression pattern and toxin sensitivity, respectively. Based on the phylogeny underlying these pharmacological and biophysical differences, Ertel et al. (2000) have suggested a more uniform nomenclature for the α1 subunit of Ca^{2+} channels which is now commonly used.

CACNA1A gene encodes the pore forming α1 subunit of P/Q type Ca^{2+} channel (CaV2.1) (Wheeler et al., 1994), and these channels are localized in high density in presynaptic active zones of central neurons (Wu et al., 1999). Ca^{2+}

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binding to calmodulin causes facilitation and enhances inactivation of *CACNA1A* channels (DeMaria et al., 2001).

Mutations of the *CACNA1A* gene result in a wide clinical spectrum varying from mild and fully reversible symptoms to deteriorative symptoms. The brain specific P/Q type Ca^{2+} channel α1A subunit gene *CACNA1A* on chromosome 19p13 has been shown to be involved in a few mainly episodic neurological disorders. Mutations in the human gene *CACNA1A* result in episodic ataxia 2, spinocerebellar ataxia type 6 and familial hemiplegic migrane (Ophoff et al., 1996). However, the association of *CACNA1A* with blood pressure regulation has not yet been examined. In the present study, polymorphisms in the *CACNA1A* genes were examined in hypertension and control individuals from a Korean population.

MATERIALS AND EMTHODS

1. Human Subject

We recruited 92 mild hypertension (BP > or = 130/ > or = 85 mmHg) and 279 control subjects at the Kyung Hee University Medical Center. All subjects were single ethnic Korean subjects. Patients with hypertension, diabetes, hyperlipidemia, stoke, and cardiac diseases were excluded. All studies were carried out according to the Declaration of Helsinki guidelines. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured, and blood samples were drawn for biochemical measurements [total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-cholesterol), fasting blood sugar (FBS), and hemoglobin A_{1c} (HbA_{1c})]. The clinical characteristics of the subjects are shown in Table 1. DNA samples were isolated from peripheral blood by G-DEX™ II b Genomic DNA Extraction Kit (iNtRON Biotechnology, Seongnam, Korea). Written informed consent was obtained from all subjects. This study was approved by the ethics committee of the Medical Research Institute, Kyung Hee University Medical Center.

2. Single nucleotide polymorphisms (SNPs) selection and genotyping

We selected 49 SNPs (rs1865030, rs12611029, rs16035, rs7249323, rs7254412, rs7251409, rs4926252, rs12608501, rs16016, rs4433935, rs11878230, rs10421428, rs2248069, rs10419230, rs11085844, rs12608866, rs1742, rs16007, rs10854124, rs7259944, rs8109003, rs10425859, rs4926283, rs752079, rs4926287, rs4632265, rs1422259, rs10419472, rs2900964, rs933649, rs11882861, rs3764615, rs1862263, rs10408880, rs7257149, rs10421810, rs4926290, rs17777900, rs7249531, rs6511867, rs2112460, rs10412211, rs1862258, rs17777941, rs7249246, rs1120559, rs10409910, rs4926294, and rs1345649) within *CACNA1A* gene using the following websites: (1) human SNP websites (http://www.ensembl.org; www.ncbi.nlm.nih.gov/SNP) (2) HapMap database (http:// www.hapmap.org) (3) tag SNPs site (http:// broad.mit.edu/ mpg/tagger). The SNPs with unknown heterozygosity and minor allele frequency (below 5%) were excluded. The genotyping was performed using the Affymetrix Targeted Genotyping Chip array (Affymetrix, CA, USA). In brief, DNA was subjected to PCR using primers specific to the adaptor sequence (P/N 900409, Affymetrix). PCR products were purified and the fragmented DNA was then end-labeled with biotin using terminal deoxynucleotidyl transferase. Labeled DNA was then hybridized onto the Mapping Array. The hybridized array was washed, stained, and scanned according to the manufacturer's instructions. The image was analyzed using GCOS software (Affymetrix).

3. Statistical analysis

Hardy-Weinberg equilibrium (HWE) for all SNPs was assessed using SNPstats (Sole et al., 2006). Multiple logistic regression models were calculated for the odds ratio (OR), 95% confidence interval (CI) and corresponding *P* values, controlling for age and gender as covariables, using SNPstats. Clinical characteristics were compared between controls and hypertension subjects, using Student's *t* test. For all statistical tests, the significant level was set at 0.05. Power analysis was performed using G*Power computer software (Faul et al., 2007).

RESULTS

We investigated whether SNPs in the *CACNA1A* genes were associated with hypertention. The characteristics of

our study population were satisfied with clinical condition of hypertension. Of the measured clinical characteristics, age, systolic blood pressure, diastolic blood pressure, BMI, triglyceride, HDL cholesterol, and fasting blood glucose differed significantly between the hypertension and control subjects, $P<0.05$ (Table 1).

Six SNPs (rs12611029, rs16035, rs7259944, rs10419472, rs17777900, and rs4926294) in *CACNA1A* gene of the 49 SNPs examined were statistically associated with hypertension (Table 2).

Table 1. Clinical characteristics of control and hypertensive subjects

Features	Control (n=279)	Hypertension (n=92)
Sex (M/F)	150/129	35/57
Age (years)	43.9±6.3*	45.7±6.1
BMI (kg/m^2)	23.3±2.7*	24.8±2.9
Systolic blood pressure (mmHg)	112.8±10.2*	142.9±15.8
Diastolic blood pressure (mmHg)	70.7±7.4*	89.0±9.7
Total cholesterol (mg/dl)	191.1±32.6	196.6±34.0
Triglyceride (mg/dl)	110.2±90.9*	156.4±112.1
HDL cholesterol (mg/dl)	53.8±12.6*	50.4±12.4
Fasting blood glucose (mg/dl)	90.2±9.8*	97.3±20.4
HbA_{1c} (%)	5.4±0.5	5.5±0.7

Data are means ± SD. BMI, body mass index. HDL cholesterol, high-density lipoprotein cholesterol. HbA_{1c}, hemoglobin A_{1c}. *$P<0.05$

The rs12611029 SNP exhibited significant association in hypertension with codominant model (P=0.008; OR, 2.00; 95%CI, 1.19~3.36), dominant model (P=0.020; OR, 1.85; 95%CI, 1.11~3.09), and recessive model (P=0.007; OR, 2.06; 95%CI, 1.23~3.45) (Table 2). The rs16035 SNP showed significant association in hypertension with codominant model (P=0.028; OR, 1.93; 95%CI, 1.17~3.17), and dominant model (P=0.017; OR, 1.80; 95%CI, 1.11~2.93) (Table 2). The rs7259944 SNP was significantly associated in hypertension with codominant model (P=0.026; OR, 3.69; 95%CI, 1.25~10.93), dominant model (P=0.022; OR, 1.78; 95%CI, 1.09~2.92), and recessive model (P=0.041; OR, 3.16; 95%CI, 1.08~9.19) (Table 2). The rs10419472 SNP was significantly associated in hypertension with dominant model (P=0.023; OR, 1.77; 95%CI, 1.08~2.88) (Table 2). The rs17777900 SNP was significantly associated in hypertension with recessive model (P=0.045; OR, 3.50; 95%CI, 1.05~11.64) (Table 2). The rs4926294 SNP was significantly associated in hypertension with dominant

Table 2. Genotype frequencies of *CACNA1A* in control and hypertensive Korean subjects

Locus	Genotype	Control	Hypertension	Codominant		Dominant		Recessive	
				OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
rs12611029	TT	205 (73.5%)	56 (61.5%)	**2.00 (1.19~3.36)**	**0.008**	**1.85 (1.11~3.09)**	**0.020**	**2.06 (1.23~3.45)**	**0.007**
	CT	67 (24%)	35 (38.5%)						
	CC	7 (2.5%)	0 (0%)						
rs16035	CC	162 (58.5%)	42 (45.6%)	**1.93 (1.17~3.17)**	**0.028**	**1.80 (1.11~2.93)**	**0.017**	0.66 (0.18~2.40)	0.510
	AC	101 (36.5%)	47 (51.1%)						
	AA	14 (5%)	3 (3.3%)						
rs7259944	CC	193 (69.2%)	51 (55.4%)	**3.69 (1.25~10.93)**	**0.026**	**1.78 (1.09~2.92)**	**0.022**	**3.16 (1.08~9.19)**	**0.041**
	CT	78 (28%)	34 (37%)						
	TT	8 (2.9%)	7 (7.6%)						
rs10419472	CC	184 (66%)	49 (53.3%)	1.57 (0.39~6.26)	0.073	**1.77 (1.08~2.88)**	**0.023**	1.25 (0.32~4.92)	0.750
	CT	86 (30.8%)	40 (43.5%)						
	TT	9 (3.2%)	3 (3.3%)						
rs17777900	GG	180 (64.8%)	52 (56.5%)	3.90 (1.15~13.19)	0.068	1.51 (0.92~2.47)	0.100	**3.50 (1.05~11.64)**	**0.045**
	AG	92 (33.1%)	34 (37%)						
	AA	6 (2.2%)	6 (6.5%)						
rs4926294	TT	77 (28.1%)	34 (37.8%)	0.66 (0.33~1.33)	0.120	**0.59 (0.35~0.99)**	**0.045**	0.94 (0.51~1.75)	0.850
	GT	145 (52.9%)	39 (43.3%)						
	GG	52 (19%)	17 (18.9%)						

OR: odds ratio; CI: confidence interval. P values were from logistic regression analyses with the codominant, dominant, and recessive models

model (P=0.045; OR, 0.59; 95%CI, 0.35~0.99) (Table 2).

DISCUSSION

Voltage-dependent Ca^{2+} channels are very important not only because of mediating the entry of calcium ions into excitable cells but because of being involved in a variety of Ca^{2+}-dependent processes, including muscle contraction, hormone or neurotransmitter release, and gene expression. Diriong et al. noted that calcium channels are multisubunit complexes and that the channel activity is directed by a pore-forming alpha-1 subunit, which is often sufficient to generate voltage-sensitive Ca^{2+} channel activity. There are at least 6 classes of alpha-1 subunits: alpha-1A, B, C, D, E, and S, which are derived from 6 genes representing members of a gene family (Diriong et al., 1995).The *CACNA1A* gene is localized in high density in presynaptic active zones of central neurons and plays an important role in the onset of idiopathic intracranial hypertension (Stanley, 2002).

In this study, we showed that genetic variations in this gene may reduce/increase the risk of hypertension. We evaluated whether the *CACNA1A* gene polymorphisms are related to hypertension by genotyping the 49 selected SNPs in the Korean population. The rs12611029 SNP is located in the intron of the *CACNA1A* gene located on chromosome 19p13.1-p13.2. The TT, CT, and CC genotype frequencies are reported, respectively, to be 0.383, 0.483, and 0.133 in European, 0.689, 0.311, and 0.000 in Chinese, 0.778, 0.222, and 0.000 in Japanese, 0.500, 0.383, and 0.117 in Sub-Saharan African (http://www.ncbi.nlm.nih.gov/SNP). In the Korean population, the TT, CT, and CC genotype frequencies were 0.735, 0.240, and 0.025 similar to those seen in Asian population, respectively. The rs16035 SNP is located in the intron of the *CACNA1A* gene located on chromosome 19p13.1-p13.2. The CC, AC, and AA genotype frequencies are reported, respectively, to be 0.350, 0.517, and 0.133 in European, 0.644, 0.267, and 0.089 in Chinese, 0.622, 0.333, and 0.044 in Japanese, 0.367, 0.433, and 0.200 in Sub-Saharan African (http://www.ncbi.nlm.nih.gov/SNP). In the Korean population, the CC, AC, and AA genotype frequencies were 0.585, 0.365, and 0.050 similar to those seen in Asian population, respectively. The rs7259944 SNP is located in the intron of the *CACNA1A* gene located on chromosome 19p13.1-p13.2. The CC, CT, and TT genotype frequencies are reported, respectively, to be 0.933, 0.067, and 0.000 in European, 0.578, 0.333, and 0.089 in Chinese, 0.756, 0.222, and 0.022 in Japanese, 0.117, 0.467, and 0.417 in Sub-Saharan African (http://www.ncbi.nlm.nih.gov/SNP). In the Korean population, the CC, CT, and TT genotype frequencies were 0.692, 0.280, and 0.029 similar to those seen in Asian population, respectively. The rs10419472 SNP is located in the intron of the *CACNA1A* gene located on chromosome 19p13.1-p13.2. The CC, CT, and TT genotype frequencies are reported, respectively, to be 0.900, 0.100, and 0.000 in European, 0.711, 0.289, and 0.000 in Chinese, 0.578, 0.356, and 0.067 in Japanese, 0.684, 0.298, and 0.018 in Sub-Saharan African (http://www.ncbi.nlm.nih.gov/SNP). In the Korean population, the CC, CT, and TT genotype frequencies were 0.660, 0.308, and 0.032 similar to those seen in Asian population, respectively. The rs17777900 SNP is located in the intron of the *CACNA1A* gene located on chromosome 19p13.1-p13.2. The GG, AG, and AA genotype frequencies are reported, respectively, to be 0.915, 0.085, and 0.000 in European, 0.689, 0.244, and 0.067 in Chinese, 0.467, 0.489, and 0.044 in Japanese, 0.950, 0.050, and 0.000 in Sub-Saharan African (http://www.ncbi.nlm.nih.gov/SNP). In the Korean population, the GG, AG, and AA genotype frequencies were 0.648, 0.331, and 0.022 similar to those seen in Asian population, respectively. The rs4926294 SNP is located in the intron of the *CACNA1A* gene located on chromosome 19p13.1-p13.2. The TT, GT, and GG genotype frequencies are reported, respectively, to be 0.117, 0.433, and 0.450 in European, 0.267, 0.533, and 0.200 in Chinese, 0.356, 0.533, and 0.111 in Japanese, 0.000, 0.067, and 0.933 in Sub-Saharan African (http://www.ncbi.nlm.nih.gov/SNP). In the Korean population, the TT, GT, and GG genotype frequencies were 0.281, 0.529, and 0.190 similar to those seen in Asian population, respectively.

Our results suggest that the five SNPs (rs12611029, rs16035, rs7259944, rs10419472, and rs17777900) and one SNP (rs4926294) in the *CACNA1A* gene have different effects in hypertension (Table 2). The rs7259944SNP was associated with 3-fold risk factor to hypertension; in contrast,

the rs4926294 was associated with 2-fold protective factor for hypertension, respectively. In our study, we have identified and confirmed that *CACNA1A* is a novel susceptible gene for the hypertensive human, and the function of *CACNA1A* in hypertension needs to be elucidated in future study.

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