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Genetic variations in *MOV10* and *CACNB2* are associated with hypertension in a Chinese Han population

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ABSTRACT. Human hypertension is a complex, multifactorial disease. Multiple variants associated with hypertension have been identified in the large numbers of genome-wide association studies, meta-analysis, and case-control studies. The present study investigated the association between the single nucleotide polymorphisms (SNPs) of five candidate genes and the susceptibility and prognosis of hypertension in a Chinese Han population. A hospital-based case-control study in a Chinese Han population was carried out, including 500 hypertension patients and 506 healthy controls. The five SNP markers were detected using the Sequenom MassArray® iPLEX System. The association of genotypes with susceptibility to hypertension was analyzed using odds

ratio, with 95% confidence interval and logistic regression. All five variants conformed to Hardy-Weinberg proportions in the controls. No significant differences were noted in the genotype distributions for *AGTR1*, *PRRC2A*, and *CALCA* polymorphisms in patients with hypertension (N = 500) and healthy controls (N = 506). SNP rs2932538, a variant in *MOV10*, was found to be significantly associated with an increased risk of hypertension. However, SNP rs4373814, a variant in *CACNB2*, showed a relevant association with a decreased risk of hypertension. In conclusion, the results of our case-control study confirmed the significant association of the SNP rs2932538 in *MOV10* and SNP rs4373814 in *CACNB2* with an increased risk of hypertension in a Chinese Han population, suggesting that the SNP rs2932538 may be a poor prognostic indicator for hypertension, while SNP rs4373814 may be a good prognostic indicator for hypertension in the same region. However, our findings need to be replicated in larger epidemiological and functional studies.

Key words: Single nucleotide polymorphism; Chinese Han population; Hypertension

INTRODUCTION

Hypertension, a leading risk factor for cardiovascular diseases and stroke, is a serious public health problem worldwide. Over one billion people worldwide have hypertension (high blood pressure), and the number of adults with hypertension was predicted to increase to 1.54-1.58 billion by 2025 (Kearney et al., 2005). In China, the statistical data from the Ministry of Health of the People's Republic of China indicated that more than 200 million people suffer from high blood pressure, thus making it the leading preventable cause of death. Therefore, it is increasingly important to exert effective measures to control the incidence of hypertension in China.

The development of hypertension appears to be determined by polygenic traits and environmental factors (Lifton et al., 2001). Although several factors, including sedentary lifestyle, constant stress situations and anxiety, high alcohol and salt intake, and lack of exercise, are known to increase blood pressure and the risk of developing hypertension (Kyrou et al., 2006), the findings in children with hypertension illustrate that genetic factors were important for the development of hypertension (Ingelfinger, 2006). Approximately 30-70% variability in blood pressure in human hypertension is attributed to multiple genetic factors (Pilia et al., 2006); monogenic disorders of hypertension are rare and do not explain the variability in blood pressure in the population at large.

The use of single nucleotide polymorphisms (SNPs) in the detection of genetic diseases is facilitated by the recent discovery of >4,000,000 SNPs in the human genome that have the potential to be a rich source of genetic markers to establish genetic linkage and as indicators of diseases (Risch and Merikangas, 1996). As SNP maps become more accessible with finer detail, there is growing recognition that rapid and efficient detection methods are required to bring SNP analysis into mainstream use in molecular diagnostic laboratories.

The use of molecular analysis to screen and detect the SNPs associated with hypertension is also recognized (Doris, 2002). To date, many genetic variants involved in the pathogenesis of hypertension have been identified by the genome-wide association studies (GWAS) (Cho et al., 2009; Levy et al., 2009; Yang et al., 2009; Ehret et al., 2011), meta-analysis (Wang et al., 2010), and case-control studies (Morita et al., 2007). Recently, 16 novel genetic loci influencing the risks of hypertension and cardiovascular disease have been identified by the International Consortium for Blood Pressure-GWAS (ICBP-GWAS) based on about 200,000 individuals of European descent (Ehret et al., 2011). Although some SNPs have been found to be associated with the development of hypertension, the association between these genetic variants and hypertension in the Chinese population is still unknown. Therefore, considering the heterogeneity among different ethnic groups, it is essential to test the genetic associations with hypertension in a Chinese Han population. In the present study, a case-control association study in a Chinese Han population of the Fujian Province was performed to evaluate the relationship between the susceptibility to hypertension and genetic variations (SNPs such as rs2932538 in *MOV10*, rs4373814 in *CACNB2*, rs805303 in *PRRC2A*, rs3781719 in *CALCA*, and rs5186 in *AGTR1*).

MATERIAL AND METHODS

Study population

We recruited 500 patients with hypertension from the Second Hospital of Fuzhou, Fuzhou, Fujian Province, P.R. China, in this study. Around 506 normal control subjects with no history of hypertension (normotension group) were randomly selected from healthy persons from the same geographic region (Fujian Province). The mean ages of the patients and control subjects were 52.25 and 40.11 years, respectively (Table 1). Patients with a history of diabetes mellitus, hyperlipidemia, liver or renal disease, congestive cardiac failure, and a recent episode of myocardial infarction were excluded. Patients with pregnancy and lactation and those receiving medications for other indications that could affect blood pressure were also excluded. All subjects involved in this study were Han Chinese, and all subjects provided informed consent before participating in the trial.

Normotension was defined as having an average systolic blood pressure (SBP) of ≤120 mmHg and a diastolic blood pressure (DBP) of ≤80 mmHg without using antihypertensive medication. Patients receiving antihypertensive medication for >3 months or newly diagnosed hypertensive patients with an SBP of ≥140 mmHg and/or a DBP of ≥90 mmHg on two or more consecutive visits were considered as hypertensives. None of the subjects in the control group was receiving antihypertensive therapy, cardiac therapy, or hormone-replacement therapy during the study. The plasma lipid profile and blood glucose levels were measured after overnight fasting in both hypertensives and normotensives to rule out diabetes and hyperlipidemia.

Genotyping

Peripheral blood samples (5 mL) were collected after obtaining written informed consent from the subjects, and delivered and stored in a frozen state. Genomic DNA was

extracted from 200 µL peripheral blood by using a Genomic DNA Purification kit (Omega, China), according to manufacturer instructions and stored at -70°C until use. All SNPs were genotyped using SEQUENOM MassARRAY matrix-assisted laser desorption ionization-time of flight mass spectrometry platform (Sequenom, USA). Primers were designed using a semiautomated method (Assay Design3.1, Sequenom). The call rate for each assay was >99%.

Statistical analysis

Hardy-Weinberg equilibrium was examined using Haploview 4.1. The associations between each SNP and hypertension were evaluated with co-dominant and dominant genetic models. Association analysis based on unconditional logistic regression was performed by calculating the odds ratio (OR) and the 95% confidence interval (95%CI) for each SNP in the co-dominant and dominant genetic models. A P value of <0.05 was considered to be statistically significant. The statistical tests were implemented in the web-based tool SNPstats (http://bioinfo.iconcologia.net/SNPstats).

RESULTS

The characteristics of the healthy controls and hypertension patients are shown in Table 1. The mean age was significantly higher in hypertensive patients (52.25 years) when compared to the control subjects (40.11 years) (P < 0.01). Hypertension was more prevalent in the old male subjects (71.54%) than in the young male subjects (62.50%). The mean blood pressure was significantly higher among hypertensive patients than among normal control subjects (151.34/95.93 *vs* 106.72/74.88 mmHg) (Figure 1 and Table 1).

Table 1. Results of 5 SNPs in hypertension cases and controls.

Nearest gene	SNP_ID	ID	Model	Genotype	Case [N (%)]	Control [N (%)]	Adjusted[a] OR (95%CI)	P[b]
MOV10	Chr1_113018066	rs2932538	Codominant	C/C	348 (70.5%)	323 (65.7%)	1	0.13
				C/T	134 (27.1%)	157 (31.9%)	1.36 (1.00-1.85)	
				T/T	12 (2.4%)	12 (2.4%)	1.33 (0.54-3.24)	
			Dominant	C/C	348 (70.5%)	323 (65.7%)	1	0.042
				C/T-T/T	146 (29.6%)	169 (34.4%)	1.36 (1.01-1.83)	
AGTR1	Chr3_149942678	rs5186	Codominant	A/A	432 (87.3%)	433 (89.7%)	1	0.64
				C/A	61 (12.3%)	49 (10.1%)	0.81 (0.52-1.27)	
				C/C	2 (0.4%)	1 (0.2%)	0.73 (0.06-9.03)	
PRRC2A	Chr6_31724345	rs805303	Codominant	C/C	190 (38.1%)	181 (36.7%)	1	0.75
				C/T	223 (44.8%)	230 (46.6%)	1.12 (0.83-1.52)	
				T/T	85 (17.1%)	82 (16.6%)	1.02 (0.68-1.53)	
CACNB2	Chr10_18459978	rs4373814	Codominant	C/C	123 (24.9%)	153 (31.2%)	1	0.067
				C/G	255 (51.7%)	227 (46.3%)	0.68 (0.49-0.95)	
				G/G	115 (23.3%)	110 (22.4%)	0.73 (0.49-1.08)	
			Dominant	C/C	123 (24.9%)	153 (31.2%)	1	0.022
				C/G-G/G	370 (75%)	337 (68.8%)	0.70 (0.51-0.95)	
CALCA	Chr11_14951100	rs3781719	Codominant	T/T	310 (66.2%)	317 (67%)	1	0.35
				T/C	139 (29.7%)	143 (30.2%)	1.08 (0.79-1.48)	
				C/C	19 (4.1%)	13 (2.8%)	0.58 (0.26-1.33)	

[a]The corresponding OR is counted by age and gender adjustment. [b]The P value is counted by the web-based tool SNPstats.

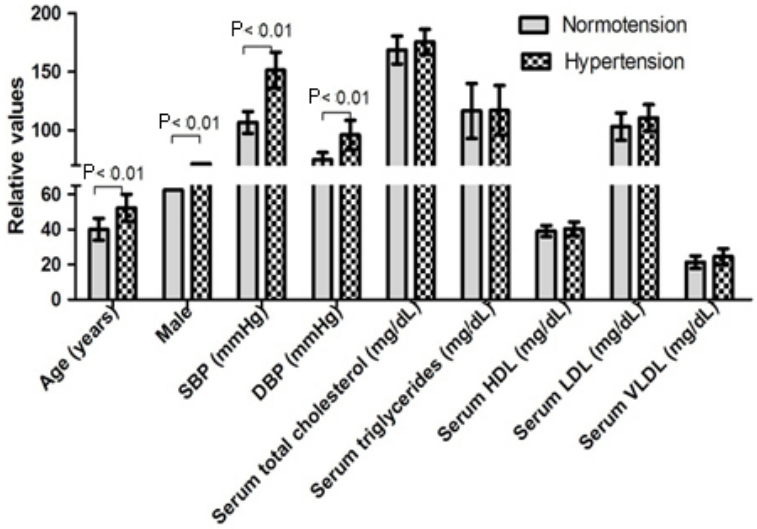

Figure 1. Biochemical parameters of the subjects studied.

To evaluate the association between genetic variations and hypertension, two factors were removed, and the genotype distributions of the five candidate SNPs (rs2932538, rs4373814, rs805303, rs3781719, and rs5186) were analyzed after adjusting for age and gender (Table 2). All five SNPs conformed to the Hardy-Weinberg proportions in the controls ($P > 0.1$; data not shown).

Table 2. Characteristics of hypertension patients and normal control subjects.

Variable	Normotension (N = 506)	Hypertension (N = 500)
Age (years)	40.11 ± 13.46	52.25 ± 15.26*
Male [% (N)]	62.50 (314)	71.54 (358)*
SBP (mmHg)	106.72 ± 9.35	151.34 ± 15.26*
DBP (mmHg)	74.88 ± 6.24	95.93 ± 12.59*
Serum total cholesterol (mg/dL)	168.6 ± 1.2	175.6 ± 1.6
Serum triglycerides (mg/dL)	116.5 ± 4.5	117.0 ± 5.3
Serum HDL (mg/dL)	39.1 ± 1.4	40.4 ± 0.96
Serum LDL (mg/dL)	103.2 ± 1.7	110.6 ± 1.3
Serum VLDL (mg/dL)	21.31 ± 0.46	24.5 ± 0.5

Values are reported as means ± SD or numbers and percentages. *$P \leq 0.01$ by the χ^2 test compared to controls.

In the present study, we noted significant differences in the genotype distributions for SNP rs2932538 in *MOV10* and SNP rs4373814 in *CACNB2* between hypertensive patients and control subjects (OR = 1.36, P = 0.042 and OR = 0.70, P = 0.022, respectively; the dominant model was the best-fit model). Adjusted by age and gender, the frequencies of C/T+T/T genotypes for *MOV10* polymorphism (rs2932538) was found to be significantly associated with increased risk (OR = 1.36, 95%CI = 1.01-1.83) of hypertension compared with the C/C genotype; the frequencies of C/G+G/G genotypes for *CACNB2* polymorphism (rs4373814)

was found to be significantly associated with decreased risk (OR = 0.70, 95%CI = 0.51-0.95) of hypertension compared with the frequency of the C/C genotype. As for the other three SNPs (rs805303 in *PRRC2A*, rs3781719 in *CALCA*, and rs5186 in *AGTR1*), no significant differences were noted in the frequencies of the genotypes for the dominant or co-dominant model between the hypertensive patients and normal controls (P = 0.75, P = 0.35, and P = 0.64, respectively).

DISCUSSION

Hypertension (elevated blood pressure levels exceeding 140/90 mmHg, according to WHO criteria) is a common complex disorder, which affects 10-20% of the adult population in mainland China (Cho et al., 2009). Blood pressure is a heritable trait influenced by several biological pathways and is responsive to environmental stimuli. Identifying gene variants that contribute to hypertension may not only improve the understanding of the pathophysiology of the disease but it may also elucidate the biochemical and physiological pathways that link various risk factors of hypertension (Ehret et al., 2011). Previous studies implicated that genetic aberrations in ion channels, ion channel regulation, aldosterone signaling, vasoconstriction, and inflammation might affect essential hypertension. Many candidate gene loci responsible for susceptibility to hypertension were linked to these pathways. In the present study, the association between five candidate SNPs and hypertension susceptibility in a Chinese Han population was investigated, four of which were located in this kind of candidate genes (rs5186 in *AGTR1*, rs805303 in *PRRC2A*, rs4373814 in *CACNB2*, rs3781719 in *CALCA*, and rs2932538 in *MOV10*).

Our results indicated that *MOV10* and *CACNB2* were associated with susceptibility to hypertension. MOV10 is a putative RNA helicase implicated in post-transcriptional gene silencing and inhibits the expression of the INK4α tumor suppressor (El Messaoudi-Aubert et al., 2010), and INK4α (p16) pathway was reported to inhibit vascular smooth muscle cell proliferation (Gizard et al., 2008). Therefore, MOV10 might play pivotal role in the regulation of vascular smooth muscle by influencing blood pressure. The association between the genetic variant of *MOV10* and hypertension susceptibility was found in our study, which was consistent with previous results (Ehret et al., 2011), and this result was observed in Asian ethnic groups. All of these studies verified the influence of *MOV10* on blood pressure. In addition, our result showed that the frequencies of C/T + T/T genotypes were associated with an increased risk of hypertension.

CACNB2 encodes the beta-2 subunit of a voltage-gated calcium channel, which is a member of the high voltage-gated calcium channel genes. High voltage-gated calcium channels consist of a pore-forming subunit [alpha(1)] and three nonhomologous subunits [alpha(2)/delta, beta, and gamma], and the beta-2 subunit could interact with alpha-1 subunit (CaV1.2) to modulate calcium channel activity; this may be the reason why variation in *CACNB2* can alter blood pressure (Lao et al., 2008). A GWAS on blood pressure and hypertension confirmed our results, and provided evidence that *CACNB2* was associated with SBP and hypertension (Luke et al., 2009). The frequencies of C/G+G/G genotypes for rs4373814 in our study were associated with a decreased risk to hypertension. Moreover, the association of the *CACNB2* polymorphism and hypertension was also observed in She ethnic minority of China based on a case-control study (Lin et al., 2011). Therefore, our present study provided additional evidence for an association between a common genetic variant and blood pressure.

However, the other three candidate genes, namely, *AGTR1*, *PRRC2A,* and *CALCA*, were not found to be associated with hypertension in the Chinese Han population after adjustment by age and gender; these findings disagree with those of previous studies. Angiotensin II receptor type-1 (AGTR1) is a G-protein coupled receptor for angiotensin II, especially in the heart and kidney, and has been studied in many health conditions, including heart failure and stroke (Henskens et al., 2007; Wu et al., 2009). *AGTR1* polymorphisms are associated with the susceptibility to hypertension and prehypertension in the American population (Mottl et al., 2008; Fung et al., 2011). A meta-analysis also found that the *AGTR1* variant has been associated with an increased risk of hypertension in the Chinese population (Levy et al., 2009). Similarly, α-CGRP (CALCA), one of the human calcitonin gene-related peptide (CGRP), was considered a risk factor for hypertension in Japanese subjects (Morita et al., 2007); PRRC2A (also known as BAT2) encodes HLA-B-associated transcript 2 was found to be associated with type I diabetes mellitus and rheumatoid arthritis, and was found to be nominally associated with an increased risk of stroke in Caucasian participants (in the Cardiovascular Health Study) (Hashimoto et al., 1999; Singal et al., 2000; Luke et al., 2009). Given the appreciable ethnic differences in the clinical presentation of hypertension, it was not surprising to observe that the same gene contributes differently to the development of hypertension in diverse populations. Considering these facts, our results were inconsistent with those obtained in a meta-analysis study involving Chinese patients with hypertension (Yang et al., 2009), thus warranting further larger epidemiological and functional studies on *AGTR1* variants to clarify this discrepancy.

To sum up, our study confirmed the association between genetic polymorphisms of *MOV10* and *CACNB2* and hypertension susceptibility in the studied subjects. In addition, rs2932538 in *MOV10* were found to be associated with poor prognosis in hypertension patients, whereas rs4373814 in *CACNB2* may be related to a good prognosis. Our findings provided important insight on studies for hypertension susceptibility genes in the Chinese population; these potential genetic variants or SNPs associated with hypertension highlight potential drug targets for the prevention or treatment of hypertension, which may lead to new therapeutic approaches, and shift the focus of management toward prevention rather than treatment. Our findings need to be replicated in further larger epidemiological and functional studies.

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