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Association of Dopamine D1 and D3 Receptor Gene Polymorphisms with Essential Hypertension in 3 Ethnic Groups in China

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Background: Essential hypertension (EH) is one of the major cardiovascular diseases. Recent studies demonstrated that dopamine D1 and D3 receptor gene polymorphisms (DRD1 and DRD3) play an important role in EH.

Material/Methods: To investigate whether DRD1 and DRD3 polymorphisms are associated with EH, 3 single-nucleotide polymorphisms (SNPs) of the DRD1 and 6 SNPs of DRD3 gene were analyzed in 3 ethnic groups. SNPStats was used to obtain odds ratios (ORs), 95% confidence intervals (CIs), and P values. Multiple logistic regression models (co-dominant, dominant, recessive, over-dominant, and log-additive) and chi-squared test were conducted to analyze the genetic data by chi-squared test.

Results: Synonymous SNPs (rs1799914 and rs4867798) of the DRD1 gene and SNPs (rs9880168) of the DRD3 were associated with EH in Hani nationality (OR 3.77, 0.63, 1.43, 5.00, respectively; 95% CI 1.05–13.54, p=0.024; 0.44–0.90, p=0.0121; 1.06–1.94, p=0.019; 1.08–23.10, p=0.017, respectively; Recessive, over-dominant model, respectively). However, none SNPs of DRD1 and DRD3 of best models showed association with EH in Han and Yi nationality.

Conclusion: These results suggest that SNPs of DRD1 and DRD3 may be contributed to essential hypertension in Hani nationality of China.

MeSH Keywords: **China • Hypertension • Polymorphism, Single Nucleotide**

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Background

Essential hypertension (EH), also called primary hypertension or idiopathic hypertension, is the form of hypertension that by definition has no identifiable cause. It is the most common type of hypertension, affecting 95% of hypertensive patients, it tends to be familial, and is likely to be the consequence of an interaction between environmental and genetic factors [1,2]. More than 50 genes associated with hypertension have been found in recent studies.

As a neurotransmitter, dopamine also serves important physiologic functions outside of the central nervous system. Activation of dopaminergic pathways prevents or mitigates the development and consequences of hypertension. The dopamine receptors are classified into “D1-like” (D1 and D5) and “D2-like” (D2, D3, and D4) based on G protein subtype coupling, with D1-like receptors coupled to Gs, which stimulates adenylate cyclases [3,4], and D2-like receptors coupled to Gi, which inhibits adenylate cyclase [5–7].

In the present study, we investigated the distribution frequencies of these 3 DRD1 polymorphic *loci* and 6 DRD3 polymorphic *loci* in a Chinese population. Since the role of DRD1 in blood pressure (BP) have been revealed in recent studies, it is the candidate gene associated with hypertension [8,9]. Association of these polymorphisms with one of the most common diseases in Chinese populations plays a critical role in diagnosis and treatment. The density of the dopamine D3 receptor subtype in circulating mononuclear cells may contribute to understanding the involvement of the peripheral dopaminergic system in hypertension [10].

People of Hani ethnicity now are widespread in China, Laos, Vietnam, and Burma. Over 90% of the Hani people live in Yunnan province of China. In China, there are about 1.65 million people of Hani ethnicity (data on the sixth national census of Chinese population: www.stats.gov.cn) [11]. The Yi ethnic minority is the sixth largest of 55 ethnic minority groups in China. They have their own script and language, which belongs to the Tibeto-Burman language group of the Chinese-Tibetan language family and includes 6 dialects [12]. Han is the largest ethnic group in China [13], and Hani and Yi are ethnic minority groups in China. However, there have been no reports about the effects of hypertension subtypes on essential hypertension among Hani and Yi people. Herein, we present the first study in the Hani, Yi, and Han ethnic groups for genetic polymorphisms of DRD1 and DRD3 among 1169 EH cases and 1171 frequency-matched unaffected controls for association with EH susceptibility.

Material and Methods

Study population

Using the WHO hypertension criteria, 1020 Han individuals, 692 people Hani individuals, and 628 Yi individuals were diagnosed with essential hypertension at the First Affiliated Hospital of Kunming Medical University and the Human Genetics Center of Yunnan University. Subjects with secondary forms of hypertension, defined as resting systolic BP ≥ 135 mmHg and/or diastolic BP ≥ 85 mm Hg, were excluded.

Determination of genotypes

We drew 5-ml peripheral blood samples from participants at the First Affiliated Hospital of Kunming Medical University and the Human Genetics Center of Yunnan University. Genomic DNA was extracted using the DNA isolation kit (Roche Diagnostics, USA), according to the manufacturer's instructions. In accordance with the known sequence of human DRD1 and DRD3, 3 primers of DRD1 and 6 primers of DRD3 were designed to amplify the entire coding region. Amplification was performed in 25- μ L reaction volume containing 1U AmpliTaq Gold-polymerase, 2 ng genomic DNA, 10 μ mol/L of each primer, and PCR assay mix (PE Applied Biosystems, Foster City, CA). All PCR fragments were carried out in 96-well plates on a programmable thermal cycler using the ABI 373 fluorescence PCR system (PE Applied Biosystems, Foster City, CA).

Statistical analysis

The χ^2 test was used to assess the EH patients and controls for differences in genotype distributions and allele frequencies. A two-tailed value of $P \leq 0.05$ was considered to be a significant difference. SNPs in Hardy-Weinberg equilibrium ($P \leq 0.05$) were analyzed by Fisher's exact test. The associations between EH and individual DRD1 and DRD3 SNPs were estimated by logistic regression analysis with adjustment for age and sex. Five genetic models (co-dominant, dominant, recessive, over-dominant, and log-additive) were chosen to evaluate the association between each SNP and EH risk. The odds ratio (OR) with 95% confidence interval (95% CI) from the genotype and allele frequency were also calculated for EH patients. We used SAS software (Version 9.1; SAS Institute Inc., Cary, NC, USA) for chi-square and Fisher's exact test and the “SNP tools in Excel” (version 1.5, Microsoft, Redmond, WA, USA) package as macro for assessing the odds ratio (OR) in all statistical analysis.

Table 1. Characteristics of the study population by sex.

	All subjects	EH	NH
Han	1020	510 (50%)	510 (50%)
Male	670 (66%)	335 (66%)	335 (66%)
Female	350 (34%)	175 (34%)	175 (34%)
Hani	692	346 (50%)	346 (50%)
Male	397 (57%)	212 (61%)	185 (53%)
Female	295 (43%)	134 (39%)	161 (47%)
Yi	628	313 (50%)	315 (50%)
Male	365 (58%)	187 (60%)	178 (57%)
Female	263 (42%)	126 (40%)	137 (43%)

The sex distribution between EH and NT was assessed.

Table 2. Characteristics of the study population by age.

	All subjects	EH	NH
Han			
Mission	0	0	0
Unique	54	52	52
Mean	53.63	53.81	53.46
Hani			
Mission	0	0	0
Unique	43	39	42
Mean	49.75	50.21	49.29
Yi			
Mission	0	0	0
Unique	40	38	39
Mean	43.48	44.04	42.92

The age distribution between EH and NT was assessed.

Results

Prevalence of hypertension

A total of 2340 unrelated people participated in this study. The prevalence of essential hypertension (EH) in the Han, Hani, and Yi ethnic populations of Yunnan province was 50% (510/1020), 50% (346/692), and 50% (313/628), respectively (Table 1). However, this was 175 (34%) for the females in the Han group, 134 (39%) in the Hani group, and 126 (40%) in the Yi group (Table 1). The mean age of the patients was 53.81 years and that of the controls was 53.46 years in the Han population, the mean age of the patients was 50.21 years and that of the controls was 49.29 years in the Hani population, and the mean age of the patients was 44.04 years and that of the controls was 42.92 years in the Yi population (Table 2).

Single-locus analysis of SNPs for essential hypertension and nonhypertension groups

Three SNPs in DRDs were chosen from the public single-nucleotide polymorphism database dbSNP (rs5326, rs1799914, and rs4867798 in DRD1; rs6280, rs32403, rs2134655, rs3773678, rs9880168, and rs3773679 in DRD3) in Hardy-Weinberg equilibrium in both EH and NH groups (Table 3).

For Han ethnicity (Table 3), we found that rs1799914(C/T) in DRD1 and rs9880168(C/T) in DRD3 were successfully genotyped in all subjects, EH, and NH, respectively (P-value=0.00029, 0.037, 0.0027; <0.0001, <0.0001, <0.0001, respectively). The remaining 7 SNPs, consisting of 2 in DRD1 and 5 in DRD3, showed deviation from the Hardy-Weinberg equilibrium expectations in the control population ($p>0.05$).

Table 3. The characteristics of the 9tSNPs in DRD1 and DRD3 by Hardy-Weinberg equilibrium.

Nationality	Loci	P-value		
		All subjects	EH	NH
Han	rs5326(A/G)	0.22	0.21	0.71
	rs1799914(C/T)	0.00029*	0.037*	0.0027*
	rs4867798(A/G)	0.044	0.021	0.66
	rs6280(A/G)	0.22	0.82	0.14
	rs32403(C/T)	1	1	0.78
	rs2134655(A/G)	0.25	0.056	0.74
	rs3773678(C/T)	0.82	0.87	0.74
	rs9880168(C/T)	<0.0001*	<0.0001*	<0.0001*
	rs3773679(A/G)	0.46	0.63	0.11
Hani	rs5326(A/G)	0.69	0.15	0.4
	rs1799914(C/T)	<0.0001*	0.00062*	<0.0001*
	rs4867798(A/G)	1	0.13	0.1
	rs6280(A/G)	0.13	0.0046*	0.57
	rs32403(C/T)	0.014*	0.013*	0.41
	rs2134655(A/G)	0.85	0.22	0.4
	rs3773678(C/T)	0.043	0.22	0.1
	rs9880168(C/T)	<0.0001*	<0.0001*	<0.0001*
	rs3773679(A/G)	0.0069*	0.037*	0.083
Yi	rs5326(A/G)	0.12	0.15	0.45
	rs1799914(C/T)	<0.0001*	0.00025*	<0.0001*
	rs4867798(A/G)	0.0011*	0.3	0.00033*
	rs6280(A/G)	0.44	1	0.25
	rs32403(C/T)	0.13	0.13	0.5
	rs2134655(A/G)	0.46	0.8	0.5
	rs3773678(C/T)	0.67	0.3	0.03*
	rs9880168(C/T)	<0.0001*	<0.0001*	<0.0001*
	rs3773679(A/G)	0.0015*	0.047*	0.013*

* $P \leq 0.05$ compared to controls (Fisher's exact test).

For Hani ethnicity subjects (Table 3), we also found that rs1799914(C/T) in DRD1 and rs9880168(C/T) in DRD3 were successfully genotyped in all subjects, EH, and NH, respectively. However, rs32403(C/T) and rs3773679(A/G) in DRD3 were successfully genotyped in all subjects and EH, respectively, and rs6280(A/G) was in EH.

For Yi ethnicity subjects (Table 3), we found that rs1799914(C/T) and rs9880168(C/T) were successfully genotyped. In addition,

rs3773679(A/G) were also successfully genotyped in all subjects, EH, and NH. rs4867798(A/G) in DRD1 was successfully genotyped in all subjects and in NH.

Associations between Individual SNPs and EH Risk

Five genetic models (co-dominant, dominant, recessive, over-dominant, and log-additive) were chosen to evaluate the association between each SNP and EH risk. Genotype frequencies

Table 4. Odds ratio (OR) for case-control study of 9 essential hypertension (EH) susceptibility loci.

Nationality	Loci	Model	Genotype	Group=EH	Group=NH	OR values (95% CI)	P-value	
Hani (n=692)	rs1799914 (n=664)	Recessive	C/C-C/T	326 (99.1%)	323 (96.4%)	1	0.024*	
			T/T	3 (0.9%)	12 (3.6%)	3.77 (1.05–13.54)		
	rs4867798 (n=682)	Recessive	G/G-A/G	247 (72.4%)	274 (80.3%)	1	0.012*	
			A/A	94 (27.6%)	67 (19.6%)	0.63 (0.44–0.90)		
	rs9880168 (n=689)	Over-dominant	G/G-A/A	185 (54.2%)	155 (45.5%)	1	0.019*	
			A/G	156 (45.8%)	186 (54.5%)	1.43 (1.06–1.94)		
	rs9880168 (n=689)			C/C	10 (2.9%)	2 (0.6%)	1	0.017*
				C/T	334 (97.1%)	343 (99.4%)	5.00 (1.08–23.10)	

* $P \leq 0.05$ (chi-squared), 95% CI – 95% confidence interval; OR – odds ratio.

of cases and controls, as well as OR and p values for the part of best-fitting genetic model (recessive and over-dominant model), are shown in Table 4. Three other models (co-dominant, dominant, and log-additive) are not shown. Significant associations between 3 of the 9 genotyped SNPs (rs5326, rs1799914, and rs4867798 in DRD1; rs6280, rs32403, rs2134655, rs3773678, rs9880168, and rs3773679 in DRD3) and EH were confirmed in 3 groups.

AG heterozygosity in rs4867798 in DRD1 was shown to be a risk factor for EH ($p=0.019$ for SNPs analyses) with $OR=1.43$ (1.06–1.94). A protective effect against EH was identified for AA of rs4867798 in DRD1 ($p=0.012$ for recessive) with $OR=0.63$ (0.44–0.90). In addition, there was a significant difference in risk factors between TT in rs1799914 in DRD1 and rs9880168 in DRD3 ($p=0.024, 0.017$), with $OR=3.77$ (1.05–13.54) and 5.00 (1.08–23.10), respectively. No significant associations with EH susceptibility between cases and controls were found for other SNPs, including rs5326 in DRD1; and rs6280, rs32403, rs2134655, rs3773678, and rs3773679 in DRD3 (data not shown). Nevertheless, no significant association between the other clinical features of EH and any genotypes was discovered in Yi and Han ethnic groups (data not shown).

Discussion

Recent studies reported that DRD1 polymorphism is associated with essential hypertension [14,15], but the Ser9Gly polymorphism in the DRD3 gene are not associated with EH in Japanese [16]. We investigated the relationship between the DRD1 and DRD3 gene polymorphism and EH in Han, Hani, and Yi populations. The results showed that the prevalence of essential hypertension (EH) in the Han, Hani, and Yi ethnic population of Yunnan province in both sexes was the same.

For subjects of Han, Hani, and Yi ethnicity, we found that rs1799914(C/T) in DRD1 and rs9880168(C/T) in DRD3 were successfully genotyped for Hardy-Weinberg equilibrium in all subjects with EH and NH. However, for ethnic minorities, DRD3 (rs32403 and rs3773679) in all subjects, EH, and rs6280(A/G) was in EH in the Hani group; rs3773679(A/G) in all subjects, EH, and NH and rs4867798(A/G) in DRD1 in all subjects and NH in Yi group were successfully genotyped.

Our study shows that the DRD1 polymorphism (rs4867798 and rs1799914) and the DRD3 polymorphism (rs9880168) were significantly associated with susceptibility to EH in Hani ethnic subjects. In addition, a protective effect against EH was identified for AA of rs4867798 in DRD1 in Hani ethnic group subjects. In addition, the DRD1 and DRD3 variant were reported to reflect genetic variation in severity of positive symptoms in acutely exacerbated schizophrenia in Han Chinese subjects [17,18]. However, we found no significant association between the other clinical features of EH and any genotypes in subjects from Han and Yi ethnic groups.

Conclusions

In summary, we investigated the DRD1 polymorphism (rs4867798 and rs1799914) and its association with susceptibility to EH in a Chinese Hani population based on a case-control study. We found that SNPs (rs9880168) of the DRD3 were associated with essential hypertension in subjects of Hani nationality, but the DRD1 polymorphism (rs4867798) for AA was a significant protective effect against EH in Hani subjects based on a case-control study. However, none of the SNPs of DRD1 or DRD3 showed an association with essential hypertension in subjects of Han and Yi nationality. Our results suggest that SNPs of DRD1 and DRD3 may contribute to essential hypertension in the Hani population in China.

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