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Association between a G894T Polymorphism of eNOS Gene and Essential Hypertension in Hani and Yi Minority Groups of China

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Background. Endothelial nitric oxide synthase (eNOS) plays an important role in maintaining blood pressure homeostasis and vascular integrity. Recently, a G894T polymorphism in exon 7 of the eNOS gene has been reported to be associated with high blood pressure. We investigated the association between this polymorphism and essential hypertension (EH) in Hani ($n = 305$ individuals) and Yi ($n = 233$ individuals) minorities of China.

Methods. eNOS genotyping with polymerase chain reaction-restriction fragment length polymorphism was performed in 267 normotensive (NT) subjects and 271 EH subjects.

Results. The frequencies of eNOS G894T genotypes in NT controls and EH cohort in Hani population were GG: 0.714 vs. 0.581; GT + TT: 0.286 vs. 0.419, respectively. The frequencies of eNOS G894T genotypes in NT controls and EH cohort in Yi population were GG: 0.552 vs. 0.717; GT + TT: 0.448 vs. 0.283, respectively. There was a significant difference in G894T genotype distribution between NTs and hypertensives in both Hani and Yi populations ($p < 0.05$).

Conclusions. The present study suggested an association between a G894T polymorphism of eNOS gene and EH in Hani and Yi minority groups of China.

Key Words: Essential hypertension, Polymorphism, eNOS, Hani minority, Yi minority.

Introduction

Genetic elements contribute to 30–50% of the blood pressure variability in human essential hypertension (EH) (1). Studies aiming at identifying contributing genes will allow us to recognize those vulnerable individuals and to classify patients in subgroups with definite genetic and pathogenic mechanisms to achieve better prevention and therapeutics. Nitric oxide (NO) produced from L-arginine by NO synthase (NOS) plays an important role in regulation of vasodilator tone and control of blood pressure (BP) in humans (2). Three isoforms of the enzyme responsible for NO formation (NOS) have been identified: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) (3,4). Some investigators demonstrated that inhibition of eNOS elevates BP in healthy humans, and disruption of the eNOS gene leads to hypertension in mice (5,6). Moreover, individuals with EH have either diminished whole-body NO production or increased inactivation leading to lower plasma levels (7,8). These results strongly implicate genetic alterations in the eNOS gene in the pathogenesis of human EH.

The human eNOS gene is located on chromosome 7, spans 21 kb, and contains 26 exons, and multiple gene polymorphisms have been reported (9). A polymorphism in exon 7 of human eNOS corresponds to a Glu-Asp substitution (Glu298Asp, also called G894T). Many researchers studied the relationship between G894T polymorphism and hypertension. There is an apparent discrepancy among results in these association studies (10–16). Moreover, the frequency of T is significantly different in the people from different ethnic groups (16,17). In our study, the distribution of eNOS 894 site and the relationship to EH have been compared in groups of EH and normortensive subjects of Hani and Yi minority groups of China.

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Subjects and Methods

Subjects

The 538 participants were aged 30−70 years. A description of these populations has been given previously (18,19). In this case-control study, an EH group was composed of 271 subjects (Hani: 172; Yi: 99) who had blood pressure (BP) ≥140/90 mmHg. In addition, subjects who were diagnosed with secondary hypertension, myocardial infarction, cerebrovascular accident or other serious diseases were excluded. The 267 subjects (Hani: 133; Yi: 134) who had BP <120/80 mmHg, no history of cardiovascular disease, diabetes mellitus or other diseases were considered as normotensive (NT) controls. Table 1 shows the characteristics of the NT and EH groups.

Genotype Determination

DNA was isolated from peripheral leukocytes with a standard phenol-chloroform method and stored at −70°C. G894T variants of the eNOS gene were detected by polymerase chain reaction-restriction enzyme digestion using the restriction endonuclease BanI (New England Biolab, Beverly, MA). Segments of eNOS were amplified from each DNA sample by PCR in 15-μL reaction volume containing 0.5 U Taq DNA polymerase (TaKaRa Taq, Dalian, China), 1X concentration of the buffer supplied, 0.2 mmol/L concentration of each deoxynucleotide triphosphate and 10 pmol of both primers (catgaggctcagcccc agaac sense and agtcaatccctttggtgctcac antisense). PCR conditions were as follows: initial denaturation at 94°C for 3 min; then 35 cycles at 94°C for 30 sec, at 59°C (annealing) for 30 sec, at 72°C (extension) for 30 sec; final extension at 72°C for 5 min. A PCR product of 206 bp was amplified, and the fragments were digested with BanI restriction enzyme (New England Biolab) by incubating at 37°C, followed by separation of the fragments on a 2.5% agarose gel, ethidium bromide stained, and analyzed with GeneGenius systems (SYNGENE, Cambridge, UK). Amplification fragments were digested by BanI into smaller fragments (125 bp and 82 bp). In the case of a G>T substitution, a BanI restriction site is lost.

Statistical Analysis

The statistical software package SPSS 11.0 (SPSS, Chicago, IL) was used. All analyses were performed separately for the two minorities. Frequencies of genotypes were studied in cases and controls by a χ^2 test. Logistic regression analysis was used to assess whether the genetic variation was associated independently with hypertension after adjustment for covariates including gender, age, and body mass index (BMI). Odds ratio (OR) was used to compare contrasts of genotypes between cases and controls. Hardy-Weinberg equilibrium was tested by χ^2 test. Numerical data were analyzed by a one-way ANOVA; $p < 0.05$ was considered statistically significant.

Results

As shown in Table 2, these frequencies of the control population were not in agreement with those predicted by Hardy-Weinberg equilibrium in Hani ($p = 0.001$) and Yi ($p = 0.005$) populations. The frequencies of eNOS G894T genotypes in NT controls and EH cohort in Hani population were GG: 0.714 vs. 0.581; GT + TT: 0.286 vs. 0.419, respectively. Allele frequencies were G: 0.801 vs. 0.738; T: 0.199 vs. 0.262. The frequency of 894G allele in NT controls and EH cohorts in Hani population were not significantly different. However, the frequency of GT + TT genotype in the NT controls was significantly lower than that in the EH group ($p < 0.05$). Compared with the GG homozygotes, T allele carriers had a significantly elevated risk of hypertension (unadjusted OR = 1.80, 95% CI 1.11−2.92; $p = 0.02$; adjusted OR = 1.76; 95% CI 1.07−2.89; $p = 0.025$). The frequencies of eNOS G894T genotypes in NT controls and EH cohort in Yi population were GG: 0.552 vs. 0.717; GT + TT: 0.448 vs. 0.283, respectively. Allele frequencies were G: 0.709 vs. 0.813; T: 0.291 vs. 0.187. There was a significant difference in

Table 1. Blood pressure of normotensive and hypertensive subjects

	Hani population		Yi population	
	Control	Hypertension	Control	Hypertension
n	133	172	134	99
Male/Female	67/66	102/70	68/66	46/53
Age (years)	50.6 ± 9.7	52.2 ± 10.5	45.7 ± 7.5	47.1 ± 10.4
BMI (kg/m^2)	21.5 ± 2.6	22.8 ± 2.8*	21.3 ± 2.7	22.7 ± 3.0*
SBP (mmHg)	104.3 ± 6.1	159.9 ± 15.7*	100.7 ± 5.8	157.2 ± 13.0*
DBP (mmHg)	69.2 ± 4.1	99.6 ± 8.6*	68.5 ± 4.2	100.1 ± 9.7*

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.
*$p < 0.01$.

Table 2. Genotype distribution and allele frequencies of normotensive and hypertensive subjects

Population		Control	Hypertension	p[a]	OR (95% CI, p) (GT + TT vs. GG)
Hani	GG	95 (0.714)	100 (0.581)		
	GT	23 (0.173)	54 (0.314)		1.80 (1.11–2.92, 0.02)[b]
	TT	15 (0.113)	18 (0.105)	0.017	1.76 (1.07–2.89, 0.025)[c]
	G	0.801	0.738		
	T	0.199	0.262	0.071	
Yi	GG	74 (0.552)	71 (0.717)		
	GT	42 (0.313)			0.49 (0.28–0.85, 0.011)[b]
	TT	18 (0.134)		0.01	0.50 (0.28–0.87, 0.015)[c]
	G	0.709	0.813		
	T	0.291	0.187	0.01	

[a]The genotypic (GT + TT vs. GG) and allelic (G vs. T) frequencies between cases and controls were compared by χ^2 test.
[b]Unadjusted OR estimated by logistic regression analysis.
[c]OR estimated by logistic regression analysis, adjusted for gender, age, body mass index.

G894T genotype distribution and allele frequency between NTs and EH in the Yi population ($p < 0.05$). Compared with the GG homozygotes, T allele carriers had a significantly decreased risk of hypertension (unadjusted OR = 0.49, 95% CI 0.28–0.85; $p = 0.01$; adjusted OR = 0.50; 95% CI 0.28–0.87; $p = 0.015$).

Discussion

It is reported that eNOS is one of the most potent metabolic determinants in humans, tonically restraining it by approximately 30 mm Hg (20). Continuously generated nitric oxide (NO) in the endothelium maintains basal vascular tone through its effect on the soluble guanylate cyclase (GS) signaling pathway and prevents leukocyte adhesion via a GS-independent mechanism (21,22). Reduction in basal NO release may, therefore, predispose to hypertension (23). Published human (24) heme domain eNOS structures revealed, indeed, that Glu298 is a part of the catalytic heme domain. It is conceivable that this site may be part of a yet-identified protein–protein interaction site (25) that is sensitive to the Glu–Asp substitution (Glu298Asp) but which does not change charge, differing by only one methylene group. A study by Tesauro et al. (26) suggests that the eNOS wild-type Glu298 and the Asp298 variant are processed differently in cells. The Asp298 variant is more prone to cleavage by naturally occurring proteases, which cleave the eNOS protein in the region of variant conservative replacement. These results indicated that the Glu298Asp (G894T) polymorphism may contribute to the complex pathogenesis of EH.

To investigate the possible implication of eNOS gene G894T polymorphism in EH, we performed a case-control study in Hani and Yi populations of China. As shown in Table 2, in the Yi minority group, the frequent GT + TT genotype and T allele in hypertensives (GT + TT: 0.283; T: 0.187) were significantly lower than that in NTs (GT + TT: 0.448; T: 0.291) ($p < 0.05$) , respectively, suggesting an association of 894G allele with EH. In contrast, in the Hani minority group, the frequent GT + TT genotype in hypertensives (0.419) was significantly higher than in NTs (0.286) ($p < 0.05$), suggesting an association of the 894T allele with EH. Hani and Yi minority groups thus showed distinct genetic determinants, and the pathogenesis of EH is likely to be different between the groups, an apparent manifestation of population specificity. Moreover, the association between the G894T polymorphism of the eNOS gene and hypertension has also been investigated by several researchers. It is reported that the 894G allele was associated with an increased risk of hypertension in Caucasians (14). In contrast, a significantly higher frequency of the 894T allele has been found to be associated with hypertension (15) and higher blood pressure levels (12) in Japanese subjects. On the other hand, no association of the G894T polymorphism has been found with either hypertension status or blood pressure levels in other populations (10,11,13,27). This inconsistency across studies has been partly attributed to lack of evaluation of possible modulating influences of subject characteristics known to differentially affect BP such as ethnicity, sex, age, and obesity (28). The biological significance of the Glu-to-Asp substitution in codon 298 of the eNOS gene locus is still unclear. The possibility exists that this polymorphism is not functional itself, but perhaps in linkage disequilibrium with a functional polymorphism in the regulatory region of the eNOS gene.

In summary, we found an association of 894T allele in eNOS gene with EH in the Hani population, but an association of 894G with EH in the Yi population. Because EH was recognized as a polygenic syndrome, further investigation in a large population on the level of gene–gene or gene–environment interaction may be necessary to confirm our results.

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