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Association of *eNOS* gene polymorphisms with essential hypertension in the Han population in southwestern China

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ABSTRACT. Endothelial nitric oxide synthase (eNOS) plays an important role in maintaining blood pressure homeostasis and vascular integrity. Polymorphisms in the *eNOS* gene have been found to be associated with hypertension in different human populations, including Northern and Southern Chinese Han populations. To examine the relationship of three *eNOS* gene polymorphisms, T-786C (rs2070744), G894T (rs1799983), and G10T (rs7830), with hypertension in the Han population in southwestern China, we carried out a study of the genotypes of three SNPs in 510 hypertensive and 510 normotensive subjects from the Yunnan Province by using PCR-RFLP and sequencing. Our SNP analyses showed that the distribution of the T-786C polymorphism did not differ between patients and controls, and that G894T and G10T

are significantly associated with hypertension in females, adjusted for covariates. Compared with the other haplotypes, haplotype H1 (TGG), carrying protective 10G and 894G alleles, significantly decreased the risk of increased essential hypertension in females, with an odds ratio of 0.68 ($P = 10^{-5}$). These results suggest that the *eNOS* polymorphism is one of the factors contributing to the predisposition for essential hypertension in the Han population in southwestern China.

Key words: Essential hypertension; *eNOS*; SNPs; Han population; Haplotype

INTRODUCTION

Essential hypertension (EH) is a major risk factor causing morbidity and mortality, in stroke, myocardial infarction, congestive heart failure, and end-stage renal disease (Lifton et al., 2001). Studies on identifying the genes involved will allow us to recognize those vulnerable individuals as well as to classify them into subgroups with definite genetic and pathogenic mechanisms, thus achieving better prevention and treatment. Endothelium-mediated vasodilatation is impaired in patients with EH. The offspring of hypertensive patients show a reduced response to acetylcholine in comparison to the offspring of normotensives. Importantly, this difference in responses is no longer statistically significant when L-arginine (a precursor of nitric oxide, NO) is co-administered with acetylcholine, indicating a putative defect of the L-arginine-NO pathway in the pathogenesis of EH (Khawaja et al., 2007). Three isoforms of the enzyme responsible for NO formation, NO synthase (NOS), have been identified: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) (Forstermann et al., 1993, 1994). It was demonstrated that disruption of the *eNOS* gene led to hypertension in mice (Haynes et al., 1993; Huang et al., 1995), while inhibition of eNOS elevated blood pressure in healthy humans.

The human *eNOS* gene is located on chromosome 7q35-q36 and contains 26 exons with a full length of 21 kb. Three polymorphic sites in the 5'-flanking region of the gene are in linkage disequilibrium (T-1474A, A-922G, and T-786C) (Wang and Wang, 2000), despite that sequences extending only to -144 bp are suggested to be essential for promoter activity (Karantzoulis-Fegaras et al., 1999). Studies using a reporter gene revealed that the T/C substitution in position -786 (rs2070744) in the promoter region reduced transcription activity of *eNOS* by approximately 50% (Nakayama et al., 1999; Miyamoto et al., 2000), perhaps due to the creation of the C allele, the binding site of protein A1 (replication repressor) (Miyamoto et al., 2000). Another polymorphism, G894T (rs1799983) in exon 7 of human *eNOS*, corresponds to a Glu-Asp substitution (Glu298Asp). It was shown from the structures of the heme domain of human eNOS that Glu298 is a part of the catalytic heme domain (Fischmann et al., 1999). It was proposed that this site may be part of a yet-to-be-identified protein-protein interaction site that is sensitive to Glu-Asp substitution (Glu298Asp) (Kone, 2000). However, the mechanism underlying the association of G894T and hypertension remains to be clarified (Tesauro et al., 2000; Fairchild et al., 2001). Polymorphism G10T (rs7830) is situated within intron 23. It was speculated that this transversion may lead to an aberrant splicing of the primary *eNOS* gene transcript

based on the fact that a 5-nucleotide motif identical to the original 5' splice site is generated (Gluba et al., 2009). However, this proposal has yet to be confirmed by the analysis of *eNOS* gene splice variants.

The results of genetic association studies of the polymorphisms in the *eNOS* gene and hypertension vary in different populations (Zintzaras et al., 2006). Many investigations have been carried out in Chinese, the largest population in the world, but reports on the relationship of the *eNOS* polymorphism and the populations in southwestern China are still scarce. In this study, the association of the three polymorphisms T-786C (rs2070744), G894T (rs1799983), and G10T (rs7830) with essential hypertension was investigated in a case-control study with a southwestern Chinese Han population.

MATERIAL AND METHODS

Subjects

A total of 1020 participants were randomly selected from both the outpatient clinic and people undergoing a medical examination in the Third People's Hospital and Kunming Yan'an Hospital of the Yunnan Province in southwestern China. All participants gave informed consent. Human sample handling was approved by the Yunnan University Ethics Committee. In this case-control led study, an EH group was composed of 510 patients with blood pressure ≥140/90 mmHg or current treatment for hypertension with antihypertensive medication. Blood pressure was measured by professionals after at least 5-min rest. Participants with diabetes mellitus, secondary hypertension, myocardial infarction, cerebrovascular accident, or other serious diseases were excluded. The normotensive group included 510 subjects with blood pressure <140/90 mmHg and without a history of hypertension. They were selected on the basis of their comparable gender and age with the hypertensive counterparts.

Genotype determination

DNA was isolated from peripheral leukocytes with a standard phenol-chloroform method and stored at -70°C. The three single nucleotide polymorphisms (SNPs) T-786C (rs2070744), G10T (rs7830), and G894T (rs1799983), which span the *eNOS* gene, were genotyped in all 1020 subjects by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Genotyping of T-786C with *Msp*I, G894T with *Ban*II, and G10T with *Bcl*I were carried out. Primers, amplification conditions and restriction enzymes for genotyping are shown in Table 1. Each 15-μL reaction system consisted of approximately 25 ng genomic DNA, 0.1 μM each primer, 200 μM dNTPs, 1.0 U Taq polymerase (TaKaRa Biotechnology Co. Ltd., Japan) and 1.5 μL 10X PCR buffer (TaKaRa). Samples were subjected to denaturation at 95°C for 5 min, followed by 37 cycles of 95°C for 30 s, 58-62°C (see Table 1 for the annealing temperature of each site) for 30 s and an extension at 72°C for 30 s, and then a final extension at 72°C for 10 min. PCR products (3 μL) were digested with 3 U restriction enzymes (see Table 1) according to manufacturer instructions. Digested fragments were analyzed by electrophoresis on a 3% agarose gel, and DNA band size was determined after ethidium bromide staining. Representative samples were selected and DNA sequences were confirmed by sequencing.

Table 1. Primer sequences, restriction enzymes and allele calling for eNOS SNPs.

SNP	Primer sequence (5'-3')	Annealing temperature	Enzyme	Allele calling (size of fragment)
T-786C (rs2070744)	5' CTGTGGACCAGATGCCAAACT 3'F 5' AGTGACGCACGCTTCCAGG 3'R	60°C	*Msp*I	T: 248 bp; C: 195, 63 bp
G894T (rs1799983)	5' CATGAGGCTCAGCCCCAGAA 3'F 5' AGTCAATCCCTTTGGTGCTCAC 3'R	62°C	*Ban*II	T: 207 bp; G: 125, 82 bp
G10T (rs7830)	5' AAAGGGGACCTGATGGAGTG 3'F 5' CCTTCAGGCAGTCCTTTGATC 3'R	58°C	*Bcl*I	C: 187 bp; A: 21, 166 bp

F = forward primer; R = reverse primer.

Statistical analysis

The SPSS 11.0 statistical software package (SPSS, Chicago, IL, USA) was used. The clinical characteristics were compared by a standard unpaired *t*-test. Logistic regression analysis was used to assess whether the genetic variation was associated independently with the hypertension after adjustment for covariates, including gender, age, body mass index, smoking, and alcohol drinking. Odds ratio (OR) was used to compare the difference of the genotypes between cases and controls. Hardy-Weinberg equilibrium was tested by the χ^2 test. Lewontin's D' (|D'|) and r2 between each pair of alleles were calculated using the SHEsis Software (http://analysis.bio-x.cn/myAnalysis.php). The SHEsis software was also used to test the potential association of statistically inferred haplotypes with hypertension by a haplotype-specific test. OR and 95%CI (confidence interval) were also calculated for each haplotype, and the Bonferroni correction was applied. $P < 0.00625$ (0.05/number of haplotypes) was considered to be significant to correct for the number of comparisons.

RESULTS

Five hundred and ten patients and 510 control subjects were enrolled in the study. Baseline characteristics of the case and control subjects are summarized in Table 2. In addition to the difference in blood pressure, hypertensive patients exhibited significantly higher body mass index and triglyceride level than control subjects (both $P < 0.001$; see Table 2). There was no significant difference in age, gender, total cholesterol level, glucose level, smoking, and drinking status between the cases and controls.

Genotype and allele distribution

Frequency distributions for the three SNPs T-786C, G894T and G10T in the population are shown in Table 3. The observed genotype distributions of T-786C and G10T were in Hardy-Weinberg (HW) equilibrium ($\chi^2 = 1.04$, $P = 0.31$; $\chi^2 = 0.86$, $P = 0.35$, respectively), while those of G894T were in HW disequilibrium ($P < 10^{-6}$). After excluding the possibility of experimental errors and further confirming the results, HW disequilibrium was confirmed. Perhaps due to the important function of the *eNOS* gene, HW disequilibrium of the *eNOS* allele has been reported in different populations (Tang et al., 2008; Serrano et al., 2010). The HW model requires several assumptions, such as an unlimited population size and that natural selection do not act on

Table 2. Baseline characteristics of the study subjects.

Parameter	Normotensive	Hypertensive	P value
Number	510	510	
Gender (male/female)	335/175	335/175	1.000
Age (years)	53.46 ± 10.44	53.81 ± 10.23	0.588
SBP (mmHg)	111.5 ± 11.09	147.92 ± 20.67	<0.001*
DBP (mmHg)	73.53 ± 7.07	94.75 ± 13.04	<0.001*
BMI (kg/m^2)	22.98 ± 2.79	24.64 ± 2.81	<0.001*
TC (mM)	5.47 ± 3.14	5.2 ± 1.14	0.073
TG (mM)	2.00 ± 1.52	2.50 ± 2.04	<0.001*
Glycemia (mM)	5.34 ± 1.35	5.48 ± 1.29	0.095
Smokers	198	193	0.748
Drinkers	135	140	0.725

Continuous variables are reported as means ± SD. SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; TC = total cholesterol; TG = triglyceride; Smokers = the number of cigarette consumers who had smoked no less than 100 cigarettes in the life history and smoked at least once during the past 30 days; Drinkers = the number of alcohol consumers who drank no less than 12 times during the year ahead of the interview. *Statistically significant.

Table 3. Distribution of genotype and allele frequencies in normotensive and hypertensive subjects and odds ratios.

SNP		Group	Allele[a]	Genotype frequency			OR [95% CI] P[b] 1/2+2/2 *vs* 1/1	Allele frequency		OR [95% CI] P[c] 2 *vs* 1
			1/2	11	12	22		1	2	
T-786C	Total	NT	T/C	466(0.914)	44(0.086)	0(0.000)	1.20 [0.78~1.85] 0.405	0.957	0.043	1.26 [0.83~1.92] 0.270
(rs2070744)		EH		458(0.898)	50(0.098)	2(0.004)		0.947	0.053	
	Male	NT		302(0.902)	33(0.099)	0(0.000)	1.02 [0.61~1.72] 0.933	0.951	0.049	1.10 [0.67~1.81] 0.712
		EH		302(0.902)	31(0.093)	2(0.006)		0.948	0.052	
	Female	NT		164(0.937)	11(0.063)	0(0.000)	1.79 [0.81~3.92] 0.148	0.969	0.031	1.74 [0.81~3.76] 0.158
		EH		156(0.891)	19(0.109)	0(0.000)		0.946	0.054	
G894T	Total	NT	G/T	367(0.720)	89(0.175)	54(0.106)	1.49 [1.14~1.96] 0.003*	0.807	0.193	1.32 [1.06~1.63] 0.012*
(rs1799983)		EH		320(0.628)	129(0.253)	61(0.120)		0.754	0.246	
	Male	NT		231(0.690)	71(0.212)	33(0.099)	1.26 [0.91~1.75] 0.172	0.796	0.205	1.18 [0.91~1.54] 0.208
		EH		208(0.621)	88(0.263)	39(0.116)		0.752	0.248	
	Female	NT		136(0.777)	18(0.103)	21(0.120)	2.27 [1.39~3.71] 0.001*	0.829	0.171	1.77 [1.20~2.60] 0.004*
		EH		112(0.640)	41(0.234)	22(0.126)		0.757	0.243	
G10T	Total	NT	G/T	235(0.461)	229(0.449)	46(0.090)	1.46 [1.13~1.89] 0.004*	0.685	0.315	1.28 [1.06~1.55] 0.010*
(rs7830)		EH		195(0.382)	262(0.514)	53(0.104)		0.639	0.361	
	Male	NT		155(0.463)	144(0.430)	36(0.108)	1.39 [1.01~1.91] 0.041	0.678	0.322	1.22 [0.97~1.54] 0.097
		EH		132(0.394)	166(0.496)	37(0.110)		0.642	0.358	
	Female	NT		80(0.457)	85(0.486)	10(0.057)	1.65 [1.06~2.57] 0.027	0.700	0.300	1.43 [1.04~1.98] 0.029
		EH		63(0.360)	96(0.549)	16(0.091)		0.634	0.366	

OR = odds ratio; 95%CI = 95% confidence intervals; NT = normotensive controls; EH = essential hypertension cases. [a]The major allele was referred to as allele 1 and the minor allele as allele 2. OR for [b]genotypes and [c]alleles, estimated by logistic regression analysis and adjusted for age, gender, body mass index, smoking, and alcohol drinking. *Statistically significant after adjusted for covariants.

the alleles under consideration. The possibility of this locus being under selection pressure has been suggested based on the case of deviation of G894→T (Glu298→Asp) substitution from HW equilibrium in patients with mild *Plasmodium falciparum* infection in an Indian population (Dhangadamajhi et al., 2009). As southwestern China, especially the Yunnan Province where our samples originated, had been one of the worst malaria-infected regions in China, the possibility that the *eNOS* G894T is under certain natural selection could not be ruled out. Nevertheless, studies with larger sample sizes as well as studies on the *eNOS* polymorphism and malaria infection may clarify this question. Since malaria and EH do not have an obviously direct cross influence, we retained G894T in our following analyses.

In T-786C, the genotype distributions were TT 91.4%, TC 8.6% and CC 0%, with the frequency of the minor allele C being 4.3% in the healthy group. Two CC bearing males identified contributed to the CC frequency of 0.4% in the patient group. Both genotype and allele frequency distribution showed that the variant is not associated with the risk of EH (see Table 3).

Genotype distribution of the G894T was GG 72.0%, GT 17.5% and TT 10.6%, with the frequency of the minor allele T being 19.3% in controls. The genotype distributions were significantly different between cases and controls. The risk of hypertension in GT and TT carriers was 1.49 times that of 894GG homozygotes (95%CI = 1.14~1.96; P = 0.003) (see Table 3). Because of gender differences in NO production (Forte et al., 1998; Tsang et al., 2001), allele and genotype frequencies of the polymorphism were analyzed separately in men and women. Stratifying the analysis by gender, we demonstrated significant associations of the genotypes GT and TT (OR = 2.27, 95%CI = 1.39~3.71; P = 0.001) and the allele T (OR = 1.77, 95%CI = 1.20~2.60; P = 0.004) of the 894 SNP and EH in women.

In G10T, the genotype distributions were GG 46.1%, GT 44.9% and TT 9.0%, with the frequency of the minor allele T being 31.5% in normotensives. The TT homozygotes and TG heterozygotes were 1.46 times more at risk of hypertension than 10GG homozygotes (95%CI = 1.13~1.89; P = 0.004). Allele T distributed more frequently in hypertensives, which contributed 1.28 times to the risk of hypertension susceptibility than allele G (95%CI = 1.06~1.55; P = 0.010). Gender stratification showed close frequencies of genotype TT (10.8 and 11.0%, respectively) and allele T (32.2 and 35.8%, respectively) in male controls and patients, but different frequencies in females between the two groups (genotype TT: 5.7 and 9.1%, respectively; allele T: 30.0 and 36.6%, respectively). The statistic value did not reach significance although there was a trend (GT + TT *vs* GG, OR = 1.65, 95%CI = 1.06~2.25; P = 0.027, and allele T *vs* G, OR = 1.43, 95%CI = 1.04~1.98; P = 0.029). A larger sample size is needed to verify the trend of EH susceptibility with 10T in southwestern Han Chinese women.

Above individual polymorphism analyses indicated that, after adjusting for covariates in a logistic regression analysis, G894T was associated with hypertension in southwestern Han Chinese women and G10T was significantly associated with hypertension in southwestern Han Chinese, possibly women only. The significance remained after the Bonferroni correction for multiple tests (0.05/3).

Haplotype analyses

Pairwise linkage disequilibrium (LD) between the three common polymorphisms was measured by the Lewontin standardized disequilibrium and the patterns of extended LD in the *eNOS* gene are shown in Figure 1. None of the SNPs were observed in significant LD.

Haplotype construction with T-786C, G894T and G10T resulted in eight haplotypes (see Table 4). Their frequencies occurring in control and patient groups are shown in Table 4. Haplotypes H1, H2 and H3 were major haplotypes (51.8, 25.1 and 14.9%, respectively, in normotensives) and haplotypes H4, H5, H6, H7, and H8 had relatively low frequencies (3.9, 1.4, 2.4, 0.4, and 0.1%, respectively, in normotensives). Compared with other haplotypes, haplotype H1 (TGG) showed a significantly lower frequency in hypertensives, demonstrating a significantly decreased risk of hypertension (OR = 0.68, 95%CI = 0.571~0.810; P = 1.49E-05). The significance remained after the Bonferroni correction for multiple tests (0.05/8). When gender was stratified, H1 was shown to contribute to lower the risk of hypertension, specifi-

cally in females (OR = 0.531, 95%CI = 0.394~0.717; P = 3.40E-05). Our results suggest a potentially protective effect in females of the *eNOS* haplotype (H1) against EH. Although haplotypes H5 and H6 showed a statistically significant difference in the distribution between the female normotensive and EH groups in Table 4, due to the very small number of the samples in the categories, a larger sample size may be needed for analysis before the association of H5 and H6 with EH in southwestern Chinese is accessed.

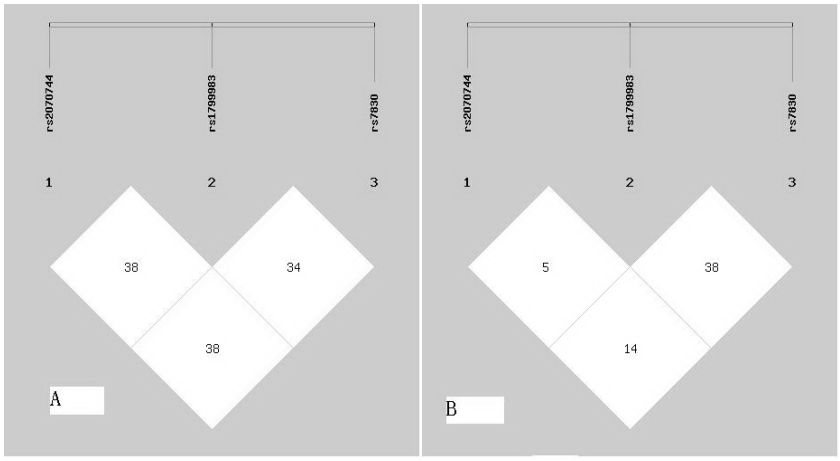

Figure 1. Pairwise linkage disequilibrium among the three polymorphisms T-786C (rs2070744), G894T (rs1799983), and G10T (rs7830) in the eNOS gene in (**A**) normotensive and (**B**) hypertensive groups.

Table 4. Association between the eNOS haplotype and essential hypertension.

Haplotype			EH	NT	χ^2	OR	[95%CI]	P
H1	TGG	Total	0.423	0.518	18.773	0.68	[0.571~0.810]	1.49E-05*
		Male	0.427	0.49	5.228	0.778	[0.627~0.965]	0.022274
		Female	0.414	0.571	17.211	0.531	[0.394~0.717]	3.40E-05*
H2	TGT	Total	0.294	0.251	4.827	1.245	[1.024~1.513]	0.028038
		Male	0.288	0.265	0.878	1.121	[0.882~1.425]	0.348811
		Female	0.305	0.227	5.353	1.489	[1.062~2.087]	0.020733
H3	TTG	Total	0.18	0.149	3.512	1.252	[0.989~1.583]	0.060955
		Male	0.187	0.164	1.286	1.177	[0.888~1.561]	0.256834
		Female	0.166	0.121	2.803	1.439	[0.938~2.205]	0.094143
H4	TTT	Total	0.051	0.039	1.686	1.322	[0.866~2.018]	0.194164
		Male	0.045	0.033	1.466	1.412	[0.806~2.474]	0.226041
		Female	0.061	0.049	0.51	1.269	[0.659~2.443]	0.475282
H5	CGG	Total	0.025	0.014	3.363	1.828	[0.951~3.515]	0.066678
		Male	0.019	0.019	0.004	0.976	[0.447~2.132]	0.9509
		Female	0.038	0.006	8.308	6.543	[1.512~28.309]	0.003964*
H6	CGT	Total	0.012	0.024	3.968	0.503	[0.252~1.002]	0.046396
		Male	0.018	0.022	0.206	0.837	[0.388~1.805]	0.650071
		Female	0	0.024	7.54	0.001	[0.000~0.021]	0.006057*
H7	CTG	Total	0.012	0.004	3.815	2.887	[0.948~8.792]	0.050797
		Male	0.008	0.005	0.575	1.698	[0.425~6.784]	0.448265
		Female	0.016	0.002	3.726	10.656	[1.335~85.084]	0.05363
H8	CTT	Total	0.004	0.001	1.393	3.201	[0.415~24.674]	0.237861
		Male	0.007	0.003	0.839	2.066	[0.423~10.096]	0.359756

SNPs are arranged in the order T-786C, G894T, G10T. *Statistical significance was established at P < 0.0063 after the Bonferroni correction. EH = essential hypertension cases; NT = normotensive controls; OR = odds ratio; 95%CI = 95% confidence intervals.

DISCUSSION

eNOS is one of the most potent metabolic determinants in humans, tonically restraining it by approximately 30 mmHg (Gamboa et al., 2007). Continuous generation of NO in the endothelium can maintain the basal vascular tone through its effect on the soluble guanylate cyclase (GS) signaling pathway and prevents the occurrence of leukocyte adhesion via a GS-independent mechanism. Reduction in basal NO release may, therefore, predispose to hypertension.

SNPs

The T/C substitution in position -786 in the promoter region is associated with a major reduction (50%) of *eNOS* transcription rate (Miyamoto et al., 2000), decreasing levels of serum nitrite/nitrate both in normal conditions and in hypoxia (Nakayama et al., 1999). Two studies could not find an association between the T-786C polymorphism and hypertension in Japanese populations (Kajiyama et al., 2000; Tsujita et al., 2001), and another population study conducted in African Americans also did not observe an association of the T-786C polymorphism with variations in the blood pressure (Li et al., 2004). In our study, no association was detected between T-786C and EH. Nevertheless, the fact that the CC genotype is very rare in the population may be due to its critical role in *eNOS* expression. Low *eNOS* expression may substantially affect metabolism and cause severe health abnormalities including but beyond blood pressure.

The G894T substitution in exon 7 is within a loop that has no contact with either the enzyme active site or the dimerization interface (Hingorani, 2001). Although its position suggests that it should not influence NOS catalysis, association of the 894G allele with increased risk of hypertension in Caucasians was reported (Lacolley et al., 1998). In contrast, a significantly higher frequency of the T allele has been found to be associated with hypertension in Japanese (Miyamoto et al., 1998; Shoji et al., 2000) and lower long-term burden of blood pressure since childhood in black females (Chen et al., 2004). Asian Indians with the 894T allele also may be at higher risk of hypertension (Srivastava et al., 2008). Contrarily, no association of the G894T polymorphism has been detected with hypertension status or blood pressure levels across a few populations from different ethnicities (Kato et al., 1999; Kishimoto et al., 2004; Zintzaras et al., 2006). In our study, G894T is associated with EH in female Hans in southwestern China. The 894T carriers, especially 894G/T heterozygotes, more likely fell in the hypertension group. A similar pattern was observed in other populations (Li et al., 2006; Srivastava et al., 2008; Tang et al., 2008). This may indicate that the influence on blood pressure from the GT heterozygous genotype is stronger than that from the TT homozygous genotype.

G10T is an intronic polymorphism site, which produces an aberrant 5' splice site. There are just a few publications addressing it, and most of them investigated its influence on the risk of hypertension (Bonnardeaux et al., 1995; Lacolley et al., 1998; Miyamoto et al., 1998; Derebecka et al., 2002; Chen et al., 2004). Some studies were not able to reveal any association between G10T and hypertension (Bonnardeaux et al., 1995; Lacolley et al., 1998; Miyamoto et al., 1998; Derebecka et al., 2002), while the 10T allele was significantly associated with lower long-term burden of blood pressure since childhood in white females (Chen et al., 2004). In our study, the T carriers showed a significant increased risk of hypertension in the Han population in southwestern China, which was likely specific to females.

Haplotypes

In this study, haplotype analysis showed that the TGG haplotype (H1) was significantly more frequent in the female normotensive group than in the EH group, suggesting that H1 was associated with normotension (OR = 0.68, 95%CI = 0.571~0.810; P = 1.49E-05). Some findings suggested a contribution of *eNOS* haplotypes to the susceptibility of hypertension, including that the haplotype 894G-10T was associated with the predisposition of hypertension in white females (Chen et al., 2004) and that two haplotypes were protective, while one increased risk, when -786, 894 and intron 4 (b/a) sites were analyzed together (Sandrim et al., 2006). In H1 (TGG) in our study, -786T was dominant over the rare and functional mutant C, and both 894G and 10G were protective alleles. The significance of H1 (P = 1.49 E-05) further confirmed the contribution of the individual SNP combination.

eNOS polymorphisms and EH in Chinese populations

The Chinese population has over 130 billion people, in which the Han population is over 117 billion. Due to variable genetic polymorphism and distinct geographic residency and life style, predisposition and susceptibility to diseases vary. With a rapidly increasing population with hypertension, a few studies have been carried out to understand the risk factors in Chinese Han (Liu and Ha, 2002; Jia et al., 2003; Tan et al., 2004; Liang et al., 2006; Zhao et al., 2006; Liu, 2009; Li et al., 2009; Zhou et al., 2010).

T-786C was suggested to be associated with the hypertension risk in northern Han in Beijing (Li et al., 2006), but no such association was detected in a northeastern (Jilin Province) Han population and near northeastern (Shangdong Province) Han population (Zhao et al., 2006). G894T is the best studied site in *eNOS* with regard to its association with hypertension in Chinese populations. A recent meta-analysis combining data from ten previous studies including northern and southern Chinese populations reported the association of G894T and EH in Chinese populations (Wang et al., 2009). Such association was not detected in the northeastern (Jilin Province) population and the near northeastern (Shangdong Province) population (Zhao et al., 2006). These results indicated that as a large and diversified population, Hans deserve close investigations with regard to *eNOS* polymorphism and hypertension.

In summary, we found that G894T and G10T, and H1 (TGG) of the *eNOS* gene were associated with essential hypertension in the female Han population in southwestern China in the present study. As essential hypertension is considered a polygenic syndrome, further investigation in a large population on the level of gene-gene or gene-environment interaction may provide further support of our findings. In addition, besides the ethnic group/population specificity (Wu et al., 2006), subpopulations in a large ethnic group/population may deserve particular investigation to clarify the role of the *eNOS* gene polymorphisms in the predisposition of essential hypertension.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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