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Polymorphisms of rs1799983 (G>T) and rs1800780 (A>G) of the eNOS gene associated with susceptibility to essential hypertension in the Chinese Hui ethnic population

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ABSTRACT. We investigated a possible association of polymorphism of the eNOS gene and essential hypertension in the Chinese Hui population; polymorphisms of rs2070744 (T>C), rs1799983 (G>T), rs1800780 (A>G), and rs3918181 (A>G) loci of the eNOS gene were examined. We found that the genotypic frequencies at rs1799983 and rs1800780 loci differed significantly between patients with essential hypertension and control cohorts. The allelic frequency of the rs1799983 locus also differed significantly between essential hypertension patients and non-essential hypertension controls in this population. Additionally, the G allele of the rs1799983 locus was less frequent in the essential hypertension patients than in controls, with an odds ratio (OR) value of 3.851 [95% confidence interval (95%CI) = 2.236-6.631]. This is an indication of a protective factor of essential hypertension in Chinese Hui people. Haplotype analysis using the 4 SNPs revealed 15 haplotypes. Haplotype frequencies of

CGAG, TTAG, TGGG, TTGG, and TTGA were significantly different in essential hypertension patients compared to non-essential hypertension controls. Individuals with haplotypes CGAG [χ^2 = 7.371, OR (95%CI) = 0.352 (0.161-0.770)] and TGGG [χ^2 = 6.180, OR (95%CI) = 0.600 (0.400-0.899)] appear less likely to have essential hypertension. However, Chinese Hui with the haplotype TTAG are at risk to develop essential hypertension [χ^2 = 10.816, OR (95%CI) = 2.689 (1.466-4.932)]. We conclude that polymorphism of the eNOS gene is associated with susceptibility to essential hypertension in the Chinese Hui population.

Key words: eNOS gene; Essential hypertension; Chinese Hui population; Polymorphisms

INTRODUCTION

Hypertension is a multifactorial disease that is influenced by both environmental and genetic factors (Colomba et al., 2008). Essential hypertension (EH) refers to high blood pressure with no obvious underlying medical cause, i.e., some unknown factors cause the high blood pressure. However, previous studies have demonstrated that genetic factors contribute to 25 to 60% of EH cases, suggesting that EH has a genetic basis (Miyamoto et al., 1998). Nitric oxide (NO) is known to be able to diffuse from the vascular endothelium to the smooth muscle cells in the vessel wall, where it activates soluble guanylate cyclase, sequentially leading to the relaxation of the vascular smooth muscle cell and to vasodilatation. Recently, increasing evidence suggests that polymorphisms of the endothelial NO synthase (eNOS) gene are associated with human EH, and certain genetic variations have been found among different ethnicities (Miyamoto et al., 1998; Benjafield and , 2000; Jachymova et al., 2001; Zhao et al., 2006; Khawaja et al., 2007; Periaswamy et al., 2008; Srivastava et al., 2008; Tang et al., 2008; Patkar et al., 2009; Li et al., 2011; Li, 2011; Men et al., 2011). eNOS is expressed in the endothelium and is encoded by a gene located on chromosome 7q35-36, comprising 26 exons that span 21 kb (Bonnardeaux et al., 1995).

The largest Chinese Hui group currently resides in Ningxia Hui Autonomous Region of China. The Chinese Hui ethnic group consists of Arabs and Persians who came to China along the Silk Road in the 13th century. To retain religious purity and group identity, the Hui have always segregated themselves socially from other people, in enclaves. Hui marriage practices tend toward endogamy in all respects, especially in the rural part of Ningxia Hui Autonomous Region. The Hui population is culturally and religiously conservative. Previous studies demonstrated the polymorphic associations of TIM genes and susceptibility of rheumatoid arthritis in the Chinese Hui minority ethnic population (Xu et al., 2011, 2012a,b). These studies revealed genetic variations in TIM genes in the loci tested between the Chinese Hui population and other ethnic groups, including the Chinese Han population (Xu et al., 2011, 2012a,b). Similarly, genetic association studies on the polymorphism of the eNOS gene and susceptibility to EH have indicated the existence of genetic variations in the eNOS gene among different ethnic populations (Benjafield et al., 2000; Chen et al., 2001; Zhao et al., 2006; Dell'Omo et al., 2007; Deng et al., 2007; Khawaja et al., 2007; Colomba et al., 2008; Periaswamy et al., 2008; Srivastava et al., 2008; Tang et al., 2008; Patkar et al., 2009; Li et al., 2011; Li, 2011; Men et al., 2011). These results may imply a genetic association of eNOS gene polymorphism with susceptibility to EH

in the Chinese Hui group. The objective of this study was to explore the correlation of the eNOS gene and susceptibility to EH in the Chinese Hui population by examining the polymorphisms of the rs2070744, rs1799983, rs1800780, and rs3918181 loci of the eNOS gene.

MATERIAL AND METHODS

Subjects

Blood samples were drawn from 134 Chinese Hui patients (76 males, 58 females) and 115 ethnically matched non-EH controls (76 males, 39 females), who were consecutively selected from the outpatient clinic of the Affiliated Hospital of Ningxia Medical University. The EH diagnosis criteria of the World Health Organization (WHO) of 1999 was used to diagnose patients with EH (Chen et al., 2012). The non-EH controls were recruited from the general Hui population and had undergone comprehensive medical screening at the Hospital. All subjects included in this study met two criteria: they were of pure Hui descent for at least three generations and had individual ancestors who had lived in the Ningxia Hui Autonomous Region for at least three generations. There was no genetic relationship between these individuals. All the samples were collected after informed consent.

Single nucleotide polymorphism (SNP) analysis

Genomic DNA was extracted from peripheral blood leukocytes using sodium dodecyl sulfate lysis and proteinase K digestion, followed by a standard phenol-chloroform extraction method (Xu et al., 2011). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed on 4 SNPs in the eNOS gene (Rahimi et al., 2012). The primer sequences and PCR parameters are listed in Table 1. PCR was performed in a 15-μL total reaction volume using 40 ng genomic DNA. The specific DNA fragment was amplified for 30 cycles at 94°C for 30 s and 57°-62°C for 30 s (Table 1), with a final extension at 72°C for 10 min, using the BioRad MyCycler Thermal Cycler (BioRad Laboratories, Hercules, CA, USA). PCR-RFLP analysis was employed for genotyping the 4 SNPs of the eNOS gene as previously described (Miyamoto et al., 1998; Benjafield et al., 2000; Hingorani, 2003; Zhao et al., 2006; Khawaja et al., 2007; Srivastava et al., 2008; Tang et al., 2008; Patkar et al., 2009; Li et al., 2011; Li, 2011; Men et al., 2011); the primer sets used for PCR and restriction endonucleases used for digestion are listed in Tables 1 and 2. For PCR-RFLP analysis, the PCR products were purified using a PCR purification kit, followed by digestion with the restriction endonuclease *Ngo*MIV, *Msp*I, *Rsa*I, and *Ban*I (Table 2). The digested PCR products were run on a 2% agarose gel containing ethidium bromide. The sizes of PCR and their corresponding digested products are listed in Tables 1 and 2, respectively. The PCR kit, PCR purification kit, and restriction endonucleases were products of Takara Biologicals (Japan).

Statistical analysis

The frequencies of genotype and allele carriers were defined as the percentage of individuals carrying the genotype and allele out of the total number of individuals. The chi-square and Fisher exact tests in SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) were used

Table 1. Primers and parameters used for PCR amplification of the eNOS gene.

Polymorphic positions	Primer sequences	Size of PCR products (bp)	Annealing temperature
rs2070744 (T>C)	F: 5'-ATGCTCCCACCAGGGCATCA-3' R: 5'-GTCCCCGAGTCTGACATTAGGG-3'	237	62°C
rs1799983 (G>T)	F: 5'-GAGATGAAGGCAGGAGACAGT-3' R: 5'-TCCATCCCACCCAGTCAAT-3'	263	62°C
rs1800780 (A>G)	F: 5'-GCATCACCAGGAAGAAGACC-3' R: 5'-CAGGATTGTCGCCTTCACTC-3'	266	58°C
rs3918181 (A>G)	F: 5'-CAACAGTGCAGGTGAATCTCA-3' R: 5'-CAAACATTACCCGCATCCT-3'	174	57°C

Table 2. Determination of the eNOS genotypes using PCR-RFLP assay.

SNP	Primer set	Restricted enzyme	Fragments of RFLP (bp)	Genotype
rs2070744 (T>C)	F/R	*Ngo*MIV	237, 204, 33 204, 33 237	CT CC TT
rs1799983 (G>T)	F/R	*Ban*II	263, 169, 94 169, 94 263	GT GG TT
rs1800780 (A>G)	F/R	*Msp*I	266, 167, 99 167, 99 266	AG GG AA
rs3918181 (A>G)	F/R	*Rsa*I	174, 150, 24 150, 24 174	AG GG AA

to test for deviation from the Hardy-Weinberg equilibrium and to compare the frequencies of discrete variables between EH patients and control individuals. The SHEsis Online haplotype analysis software (http://analysis.bio-x.cn/myAnalysis.php) was applied. $P < 0.05$ was considered to be statistically significant.

RESULTS

Four SNPs in the eNOS gene and their associations with susceptibility to EH in different populations have been previously investigated (Miyamoto et al., 1998; Benjafield et al., 2000; Hingorani, 2003; Zhao et al., 2006; Khawaja et al., 2007; Srivastava et al., 2008; Tang et al., 2008; Patkar et al., 2009; Li et al., 2011; Li, 2011; Men et al., 2011). To determine if these SNPs were also associated with susceptibility to EH in the Chinese Hui population, we analyzed the polymorphism of the eNOS gene at the loci rs2070744 (T>C) (also referred to as T786C), rs1799983 (G>T) (also referred to as G94T), rs1800780 (A>G), and rs3918181 (A>G) in 134 EH patients and 115 non-EH controls from the Hui population using a PCR-RFLP assay.

Following *Ngo*MIV, *Msp*I, *Rsa*I, and *Ban*I digestion of the PCR products at the 4 polymorphic sites, respectively, all three genotypes were determined for each SNP: rs2070744 site: (TT, CT, and CC); rs1799983 site (GG, GT, and TT); rs1800780 site (AG, GG, and AA); and rs3918181 site (AA, GG, and AG) (Figure 1A-D). Statistical analysis demonstrated a significant difference in the genotype and allele frequencies of rs1799983 (G>T) (G894T) between EH patients and controls of Hui ethnicity ($P > 0.05$, Table 3). This result was consistent with the findings of previous studies in other populations (Benjafield

et al., 2000; Chen et al., 2001; Zhao et al., 2006; Khawaja et al., 2007; Tang et al., 2008; Li, 2011; Men et al., 2011). Interestingly, a statistical difference in the genotype of rs1800780 locus was first found between the EH patients and control cohorts in the Chinese Hui population, where the genotype of AG was more frequent in the EH patients ($P > 0.05$, Table 3). However, in agreement with the study in Japanese groups, there was no significant difference in the polymorphisms of the rs2070744 (T>C) (T786C) and rs3918181 (A>G) sites between the EH patients and non-EH controls in this ethnic group ($P > 0.05$, Table 3) (Kajiyama et al., 2000). The G allele of the rs1799983 (G>T) (G894T) SNP was significantly less common in EH patients than in controls ($P < 0.05$) [OR = 3.851, 95% confidence interval (95%CI) = 2.236-6.631], while the T allele of this SNP was significantly more common in EH patients than in controls ($P < 0.05$) (OR = 0.260; 95%CI = 0.151-0.447) (Table 3). This finding suggests that individuals with the G allele were less likely to have EH, while those with T allele were at risk for EH in the Chinese Hui population.

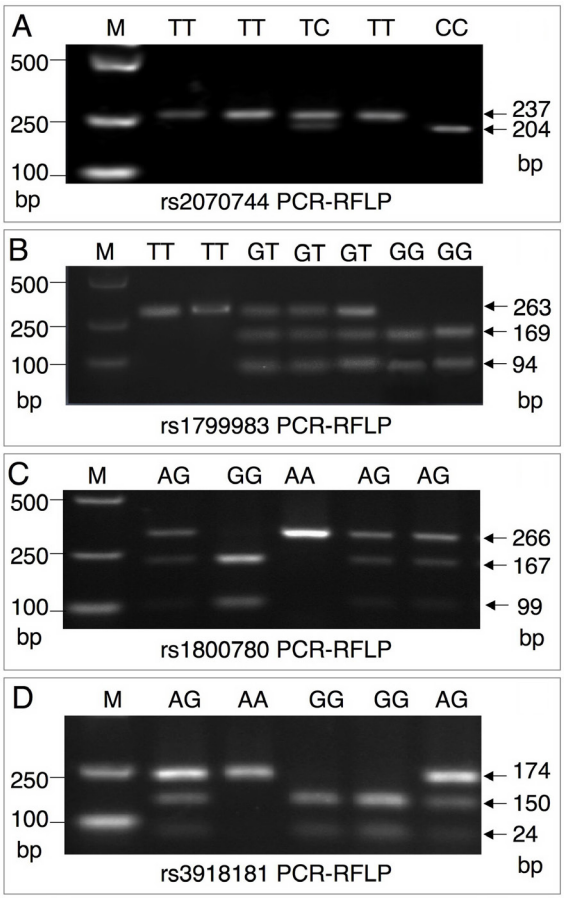

Figure 1. Genotype analyzed by polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). The PCR amplified products were digested with **A.** *Ngo*MIV (for rs2070744), **B.** *Msp*I (for rs1799983), **C.** *Rsa*I (for rs1800780), and **D.** *Ban*I (for rs3918181) before they were resolved on an agarose gel. *Lane M* = DNA molecular ladders. The rest of the lanes indicate the corresponding genotypes labeled at the top of each picture.

Table 3. Genotype and allele analyses of polymorphisms of the eNOS gene in essential hypertension (EH) patients and non-EH controls of the Chinese Hui population.

Position	Genotype/allele	Control [N (%)]	EH [N (%)]	χ^2	P	OR (95%CI)
rs2070744	TT	88 (76.5)	116 (86.6)	5.198	>0.05	-
	CT	24 (20.9)	14 (10.4)	-	-	-
	CC	3 (2.6)	4 (3.0)	-	-	-
	C	30 (13.0)	22 (8.2)	3.094	>0.05	0.596 (0.334-1.066)
	T	200 (87.0)	246 (91.8)	-	-	1.677 (0.938-2.998)
rs1799983	GG	97 (84.3)	70 (52.2)	28.961	<0.05	-
	GT	17 (14.8)	59 (44.0)	-	-	-
	TT	1 (0.9)	5 (3.7)	-	-	-
	G	211 (91.7)	199 (74.3)	26.012	<0.05	3.851 (2.236-6.631)
	T	19 (8.3)	69 (25.7)	-	-	0.260 (0.151-0.447)
rs1800780	AA	20 (17.4)	19 (14.2)	6.422	<0.05	-
	AG	56 (48.7)	86 (64.2)	-	-	-
	GG	39 (33.9)	29 (21.6)	-	-	-
	A	96 (41.7)	120 (45.5)	0.690	>0.05	1.163 (0.814-1.662)
	G	134 (58.3)	144 (54.5)	-	-	0.860 (0.602-1.228)
rs3918181	AA	15 (13.0)	19 (14.2)	0.482	>0.05	-
	AG	40 (34.8)	51 (38.1)	-	-	-
	GG	60 (52.2)	64 (47.7)	-	-	-
	A	70 (30.4)	89 (33.2)	0.438	>0.05	1.136 (0.778-1.660)
	G	160 (69.6)	179 (66.8)	-	-	0.880 (0.602-1.285)

OR = odds ratio; 95%CI = 95% confidence interval.

We next analyzed the haplotypes of the eNOS gene in EH patients and controls from the Chinese Hui population using the SHEsis Online haplotype analysis software. Fifteen of the haplotypes were detected in the samples studied (Table 4). Statistically significant differences in haplotype frequency distribution for the haplotypes CGAG, TTAG, TGGG, TTGG, and TTGA were observed between the EH patients and controls ($P < 0.05$). Haplotypes of CGAG and TGGG (bold and italics in Table 4) [$\chi^2 = 7.371$ and 6.180, OR = 0.352, 95%CI = 0.161-0.770 and 0.600 (0.400-0.899), respectively] were less common in EH patients, while haplotype TTAG (bold and underlined in Table 4) ($\chi^2 = 10.816$, OR = 2.689, 95%CI = 1.466-4.932) was more common in the EH patients, in comparison with the non-EH controls ($P < 0.05$).

Table 4. Frequencies of haplotype of eNOS SNPs in EH patients and non-EH controls in the Chinese Hui population.

Haplotype				Frequencies (%)[a]		χ^2	P[b]	OR (95%CI)[c]
rs2070744	rs1799983r	s1800780	rs3918181	EH	Control			
C	***G***	***A***	***G***	0.036	0.097	7.371	<0.05	0.352 (0.161-0.770)
C	T	A	G	0.016	0.005	-	-	-
C	G	A	A	0.000	0.007	-	-	-
C	T	A	A	0.000	0.009	-	-	-
C	G	G	G	0.000	0.013	-	-	-
C	G	G	A	0.028	0.000	-	-	-
T	G	A	G	0.204	0.190	0.295	>0.05	1.132 (0.724-1.768)
T	**T**	**A**	**G**	0.161	0.069	10.816	<0.05	2.689 (1.466-4.932)
T	G	A	A	0.034	0.041	0.134	>0.05	0.841 (0.331-2.131)
T	***G***	***G***	***G***	0.217	0.322	6.180	<0.05	0.600 (0.400-0.899)
T	G	G	A	0.224	0.248	0.227	>0.05	0.903 (0.595-1.372)
C	T	G	A	0.002	0.000	-	-	-
T	T	A	A	0.012	0.000	-	-	-
T	T	G	G	0.034	0.000	8.177	<0.05	-
T	T	G	A	0.032	0.000	7.724	<0.05	-

[a]Values were constructed by EM algorithm with genotype SNPs; [b]Values were analyzed by permutation test; [c]Values were analyzed by the χ^2 test from a 2 x 2-contingency table. OR = odds ratio; 95%CI = 95% confidence interval. Letters in bold and italics = haplotype of CGAG and TGGG. Letters in bold and underlined = haplotype of TTAG.

These data indicated that individuals of Chinese Hui ethnicity with haplotypes CGAG and TGGG may have a lower likelihood of developing EH, while those with haplotype TTAG are at risk for EH.

DISCUSSION

EH is a complex disease with unknown causes involving risk factors related to both genetics and the environment. A large body of studies show evidence that genetic factors are key risk factors of EH susceptibility in various ethnicities. In this study, the association of eNOS gene polymorphism with EH in the Chinese Hui population was investigated. The results presented in this report revealed that the polymorphisms of rs1800780 (A>G) and rs1799983 (G>T) of the eNOS gene were associated with susceptibility to EH in the Chinese Hui ethnic population. However, the polymorphisms of rs2070744 (T>C) and rs3918181 (A>G) in the eNOS gene lacked any association with EH in this ethnic group.

eNOS is considered the main source of NO in the vascular endothelium (Cau et al., 2012), in which eNOS diffuses into vascular smooth muscle cells and activates guanylate cyclase, leading to vasodilatation (Jachymova et al., 2001). An increasing number of genetic studies have demonstrated that DNA polymorphisms in the eNOS gene are associated with coronary artery disease, such as hypertension (Senthil et al., 2005). Previous polymorphic studies revealed a positive association of rs1799983 (G>T) (G894T mutation) of the eNOS gene with susceptibility to EH in various ethnic groups (Chen et al., 2001; Zhang et al., 2006; Khawaja et al., 2007; Tang et al., 2008; Li, 2011; Men et al., 2011; Niu and Qi, 2011). In contrast, the rs2070744 (T>C) variant (T786C mutation) was reported to lack an association with EH in Chinese Kazakh and Japanese groups (Kajiyama et al., 2000; Deng et al., 2007). Consistent with these findings in other ethnic populations, such as Chinese Han (Niu et al., 2011; Chen et al., 2012), Hani (Tang et al., 2008), Yi (Tang et al., 2008), and Uygur (Zhang et al., 2006) in China, polymorphism of rs1799983 (G>T) in the eNOS gene was found to be a risk factor of EH, while rs2070744 (T>C) polymorphism lacked an association with EH, in the Chinese Hui ethnic population in this study. Notably, a positive correlation between rs1800780 (A>G) polymorphism and susceptibility to EH was determined here in the Chinese Hui group. The AG genotype was more frequent in the EH patients in comparison with the controls in this population ($P < 0.05$). This finding provides evidence for the first time that the polymorphism of the rs1800780 (A>G) site is associated with EH, which expands the list of risk loci for EH.

In summary, we found that the polymorphisms of rs1799983 (G>T) and rs1800780 (A>G) in the eNOS gene, but not of rs2070744 (T>C) and rs3918181 (A>G), produce potential genetic variants in EH in the Chinese Hui population. Furthermore, the rs1799983 (G>T) polymorphism is associated with EH susceptibility in this population. Individuals of this ethnicity with haplotype TTAG are at risk for EH, and those with haplotypes of CGAG and TGGG may have a decreased likelihood of developing EH.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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