

Association between the variable number of tandem repeat polymorphisms of endothelial nitric oxide synthase and ischemic cerebrovascular diseases in Henan Han ethnicity

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Abstract

Objective. To investigate the association between the variable number of tandem repeat polymorphisms of endothelial nitric oxide synthase and ischemic cerebrovascular diseases in the Henan Han ethnicity. **Methods.** The genotypes of 488 cases with ischemic cerebrovascular diseases and 420 healthy subjects were detected by PCR. **Results.** Three genotypes, namely 5/5 repeats homozygous, 5/4 repeats heterozygous, 4/4 repeats homozygous were identified. There was a significant difference in the frequencies of eNOS VNTR mutations and the frequencies of alleles between the test group and the control group. There was a significant difference in the frequencies of eNOS VNTR mutations and the frequencies of alleles between the other ethnicities and the Henan Han ethnicity. The frequencies of alleles of eNOS VNTR are higher in the Henan Han ethnicity than those in the other ethnicity. **Conclusion.** eNOS VNTR gene mutations may be a risk factor for ischemic cerebrovascular diseases in the Henan Han ethnicities. The prevalence of the eNOS 4a/b polymorphisms varies with different ethnic groups. [Life Science Journal. 2007; 4(3): 26 – 29] (ISSN: 1097 – 8135).

Keywords: ischemic cerebrovascular diseases; eNOS VNTR; polymorphism

1 Introduction

The ischemic cerebrovascular diseases (ICVD), which have high incidence, mortality, and disability, are common and serious diseases and now have become one of the three most fatal diseases in the world. Some new researches have indicated that ICVD are related with both hereditary and environmental factors. Recently, many researchers have focused on the predisposing genes of the related risk factors of cerebral infarction. Thus the eNOS gene is considered to be an important gene for ICVD.

Nitric oxide synthase (NOS) catalyzes the 5 electron oxidations of the amino acid L-arginine to form L-citrulline and nitric oxide (NO)^[1]. The three forms of NOS have been identified: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS)^[2]. eNOS is constitutively expressed in endothelium and serves to maintain

basal NO production which regulate vascular tone and plate aggregation. The human gene encoding eNOS is located in chromosomal region 7q35-36 and composed of 26 exons that span 21 Kb^[3]. The gene exhibits a number of polymorphic repeats that have been used for the analysis of genetic association with various human cardiovascular disorders. Among them the 27 bp repeat, a variable number of tandem repeats (VNTR) genetic marker in intron 4 of eNOS gene, are believed to have importance on the occurrence of essential hypertension^[4], myocardial infarction^[5,6] and venous thrombus^[6-8], but its role for the gene variants in the risk of ischemic stroke is controversial. It has been noticed that these gene mutations have heterogeneous distributions among different ethnic groups or geographic areas. The data on the prevalence of the gene mutations in Henan Han ethnicity is not yet available.

To define the importance of eNOS 27 bp repeat polymorphism in the Henan Han ethnicity with ischemic cerebrovascular diseases, we have investigated the prevalence of eNOS4, 27 bp repeat among Henan Han ethnicity,

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which consisting of 488 patients with ICVD of the test group, and 420 healthy subjects of the control group and aimed at exploring the association between the variable number of tandem repeat polymorphisms of endothelial nitric oxide synthases and ischemic cerebrovascular diseases in the Henan Han ethnicity.

2 Materials and Methods

2.1 Subjects

A total of 488 patients with ICVD from unrelated kindred of Chinese Han ethnicity in Henan area were admitted to the Hospital of Henan province from December 2004 to July 2006, and recruited in this research. In the ICVD group, there were 298 males and 190 females, with an average age of 60 ± 10.2 years old. They were diagnosed of ICVD by clinical symptoms and CT or MRI scan. Control group included 420 healthy individuals (234 males and 186 females), with an average age of 56 ± 9.8 years old. All the subjects in the research were of the Chinese Han ethnicity. Severe systemic diseases such as, neoplastic, liver or renal diseases were excluded out of both test group and control groups. This research was approved by the Ethic Committee of the hospital and written consents were provided to each patient and health individuals.

2.2 DNA extraction

Blood samples were drawn from the patients and controls in the fasting state and collected in EDTA tubes. DNA was extracted with phenol/chloroform extraction procedure from peripheral blood leukocytes.

2.3 DNA was amplified by PCR

The primers and the experimental conditions were used to detect the eNOS 27 bp repeat polymorphism by PCR as previously described^[9]. The sequence of primers are as follows: forward: 5'-AGGCCCTATGGTAGTGCCTTT-3' and reverse: 5'-TCTCTTAGTGCTGTGGTCAC-3'. The amplification included thirty-five cycles (94 °C for 60 seconds, 58 °C for 60 seconds, and 72 °C for 60 seconds). The amplification products were determined by 2.5% agarose gel electrophoresis and stained with 0.5 g/mL of ethidium bromide, and visualized under UV light.

2.4 Statistical analysis

Statistical analysis was performed by the SPSS 11.0 for Windows statistical package. Genotype frequencies were compared between the cases and controls by using chi-square test. The relative risk of genotypes and alleles was described by odds ratios (OR) and 95% confidence intervals (95% CI). Hardy-Weinberg equilibrium was

confirmed with chi-square test. *P* values were two-tailed, and statistical significance was accepted as $P < 0.05$.

3 Results

3.1 Identification of PCR products

The products synthesized by T7 promote polymerase were separated by electrophoresis. Results indicated that the quality of shRNA-NS was satisfying, with only one band in lane 1 and lane 2 respectively, illustrating no degradation, no diffusion, and the complete removal of DNA templates. The molecule sizes of shRNA-NS were 49 bp (Figure 1).

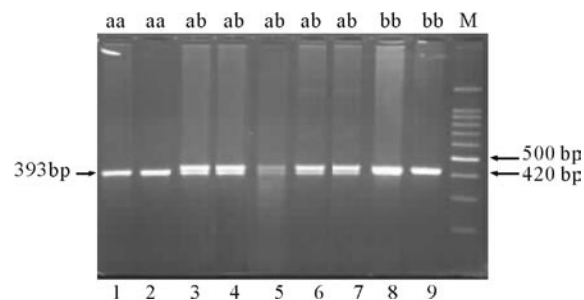


Figure 1. Genotype of the VNTR in intron 4 of eNOS gene. Lane 1, 2: 4/4 repeats homozygous; Lane 3, 4, 5, 6: 4/5 repeats heterozygous; Lane 8, 9: 5/5 repeats homozygous.

Three genotypes, containing 4, 5 repeats namely 4/4-homozygous (4aa), 4/5-heterozygous (4ab), 5/5-homozygous (4bb), were identified in the Henan Han ethnicity. The allele containing five of 27 bp repeats gave rise to a PCR products of 420 bp consisted of 135-bp repeats and 285 bp flanking sequences; whereas the four of such repeats yielded a 393 bp products consisting of 108 bp repeats and the same flanking sequences.

3.2 Distribution of the eNOS 4a/b polymorphisms

The distribution of the eNOS 4a/b polymorphisms in the test group and control group was shown in Table 1.

The distribution of genotype frequencies in the total study population was in agreement with the Hardy-Weinberg equilibrium. eNOS 4a/b of genotype and allele distribution in test group were obviously different from control group.

The relative risk of eNOS 4a/b gene a-allele carriers was 1.546 (Table 2). Relative risk for ischemic stroke in patients who had homozygote (aa) and heterozygous (ab) for eNOS 4a/b gene mutations was higher than the control group.

Table 3 showed that the differences in genotype and allele frequencies between the Henan Han ethnicity and the other ethnicities. In the Han ethnicity, the frequencies of the

Table 1. The distribution of the eNOS 4a/b polymorphisms in the random and control groups

Groups	n	Frequencies of genotypes (n, %)			Frequencies of alleles (n, %)	
		bb	ab	aa	b	a
Patient	488	387 (79.3)	90 (18.4)	11 (2.3)	864 (88.5)	112 (11.5)
Control	420	359 (85.48)	57 (13.57)	4 (0.95)	775 (92.3)	65 (7.7)
			$\chi^2 = 6.671, P = 0.036$		$\chi^2 = 7.168, P = 0.007$	

Table 2. The relative risk of eNOS 4a/b gene mutations in patients with ICVD

Groups	n	Frequencies of genotypes (n, %)			Frequencies of alleles (n, %)	
		bb	ab	aa	b	a
Patient	488	387	90	11	88.5	11.5
Control	420	359	57	4	92.3	7.7
OR	1.0	1.465	2.251	1.0		1.546
95% CI			1.020 – 2.103	0.805 – 8.084		1.122 – 2.130

Table 3. The genotype and allele frequencies of 27 eNOS 4a/b gene in difference races

	Genotypes				Alleles		
	bb	ab	aa	Total	a	b	Total
Chinese	359 (85.48)	57 (13.57)	4 (0.95)	420	65 (7.7)	775 (92.3)	840
Italian	385 (71.7)	138 (25.7)	14 (5.00)	537	166 (15.46)	908 (84.54)	1074
Korean	217 (78.62)	54 (19.57)	5 (1.81)	276	64 (11.59)	488 (88.4)	552

aa + ab genotypes were significantly lower than that of Korean ($\chi^2 = 5.482, P < 0.05$) and Italian ($\chi^2 = 25.69, P < 0.01$). The a-allele in Han ethnicity was also lower than that in Korean and Italian ($\chi^2 = 8.357, P < 0.05$, comparing with Korean; $\chi^2 = 26.458, P = 0.000$, comparing with Italian). Significant differences were found in all of genotype and allele frequencies between the Henan Han ethnicity and Italian or Korean.

4 Discussion

The NO genes are reasonable candidates to be potentially associated with ischemic cerebrovascular diseases. It has been recently reported that the a/b homozygous of the 4a/b eNOS polymorphism have shown an association with ischemic cerebrovascular disease, while the other studies did not confirm this result. The purpose of the present research was to examine the association between the eNOS-4a/b polymorphism and ischemic cerebrovascular diseases in the Henan Han ethnicity.

Our results are coincided with those of Elbaz^[10] who examined 460 individuals with ICVD and 460 healthy subjects and found a significant difference in the a-allele frequencies between healthy subjects and ICVD patients.

Furthermore, our results are also accordance with those of Hou^[11] who examined 364 ICVD patients and found a higher risk of ICVD compared with non-carriers and there were significant differences between the test group and control groups. However, Markus^[12], showed in a study of English population (361 patients and 236 healthy individuals) that, there was no relationship between the polymorphisms of eNOS-4a/b and ICVD. Yahashi^[13] also found no difference between the presence of the eNOS-4a/b polymorphism and ICVD in the Japanese population of the 127 ischemic stroke patients and 91 healthy subjects.

We have identified three genotypes and two alleles for the 27 bp repeat in intron 4 of eNOS gene in the Henan Han ethnicity. It was demonstrated that the frequencies of both genotype and allele for this VNTR in Chinese differed significantly from Italian and Korean suggesting that the distribution of this genetic marker differs among the ethnicities. Our data indicate that the distribution discrepancy may be attributed to the ethnicity diversity. These specific eNOS variants are expressed variably in different subgroups, based on a variable interaction with other unknown genetic and environmental factors. What kind of people with this VNTR polymorphism would be associated with cardiovascular disorders in Chinese population needs to be determined in further researches.

5 Conclusion

Our research finds the association between the eNOS 4a/b polymorphism and ischemic cerebrovascular diseases that can not confirm the rescent findings of Matyar^[14]. The possible reasons for this discrepancies between the studies may be the differences in the distribution of the eNOS genotype in various population selection factors and ethnicity.

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