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Association of the 894 G>T polymorphism in the endothelial nitric oxide synthase gene with risk of venous thromboembolism in Chinese population

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ABSTRACT

Introduction: Endothelium derived nitric oxide (NO) is a key mediator of vascular homeostasis. Endothelial nitric oxide synthase (eNOS) gene, by affecting the expression and functional activity of the eNOS enzyme, thereby reducing NO availability, may be implicated in venous thromboembolism (VTE). We investigated the eNOS G894T polymorphism in VTE patients in the Chinese population.
Materials and methods: A case-control study was conducted in a general hospital. Blood samples, collected from 462 consecutive patients with VTE and 462 healthy controls, were used for DNA extraction. Single nucleotide polymorphisms (SNP) of eNOS (894 G/T) were determined by allele specific-polymerase chain reaction (ARMS-PCR) analysis.
Results: The eNOS 894 G/T polymorphism alleles distribution was in agreement with the principle of Hardy-Weinberg equilibrium. The prevalence of homozygote, heterozygote and pathological homozygote for the eNOS G894T polymorphism in VTE patients was 79.7%, 18.1% and 2.2%, respectively (controls: 86.6% 12.3% and 1.1%). T allele distribution in the VTE (11.3%) and especially the male VTE patients (12.5%) was more common than in healthy controls (7.3%). The frequency of GT + TT genotype was significantly higher among the age ≤ 55 years patients in VTE group than in controls (20.1% vs. 12.2%, $P = 0.033$).
Conclusion: Our result demonstrates that the 894 G/T polymorphism variant of eNOS is a risk factor for VTE in Chinese population.

Introduction

Endothelium derived nitric oxide (NO) is critical to vascular homeostasis. NO has potent vasodilator and anti-proliferative effects, as well as antithrombotic properties, e.g. it stimulates smooth muscle cell relaxation and inhibits platelet aggregation and leukocyte adhesion [1]. Loss of NO bioavailability is associated with risk factors for atherosclerotic/ thrombotic diseases. Endothelial nitric oxide synthase (eNOS) is one of three isoforms of nitric oxide synthase [2]. Polymorphism of the eNOS gene G894T has been shown to altering the primary structure of the eNOS enzyme [3] as well as other components of the eNOS gene and/or enzymatic stability, and affecting the synthesis of NO [1]. Among several other polymorphisms in the eNOS gene that have been described, only the 894 G>T substitution involves an amino acid substitution (Glu298Asp). The 894 G > T variant has been reported to be associated with increased risk of coronary artery disease and the death after coronary stenting[4–8].

Venous thromboembolism (VTE) refers to deep venous thrombosis (DVT) complicated with or without pulmonary embolism (PE). It is a multicausal disease and involves not only genetic but also acquired risk factors such as immobilization, surgery, pregnancy and use of oral contraceptives. The most recognized Factor V Leiden mutation causes resistance to activated protein C [9]. The association between two expressive polymorphisms of the eNOS gene (intron4, 27 bp repeat and 2786 T/C) and the risk of VTE has been investigated in the Caucasian population, a weak association between these eNOS polymorphisms and VTE was reported [10]. The reports on the association between the eNOS G894T polymorphism and diseases were inconsistent and most of the studies were conducted in Caucasian populations with cardiovascular disease [4–8]. To date, there is very limited knowledge of this polymorphism in association with VTE. In this study, we investigated the eNOS G894T polymorphism and the relationship of

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this polymorphism to the pathogenesis and development of VTE in Chinese population.

Methods

Study population

462 consecutive patients, admitted in Beijing Chao-Yang hospital with the diagnosis of VTE were enrolled in the study. All of the study subjects were interviewed by a physician. The medical history, risk factors, symptoms and clinical signs were collected. Color duplex imaging of the common femoral, superficial femoral, popliteal and proximal greater saphenous veins was performed. DVT was confirmed if there was noncompressibility of the vein in combination with at least one of the following: an enlarged vein, a hypoechoic lumen, or absence of collaterals [11,12]. PE was confirmed by spiral CT pulmonary angiography and/or high probability of ventilation-perfusion lung scan. The date of occurrence and the site of every episode of VTE were also collected. 462 healthy volunteers were recruited from the same geographic area as were the patients. The healthy controls had no history of VTE or clinical evidence of VTE and were matched by age(±2 y), sex, ethnicity origin, and residence area. The study was approved by the local ethics committee. After written informed consent, blood samples were obtained from all of the study participants.

Genetic analysis

Blood samples of the participants were genotyped for the 894 G>T polymorphism. Genomic DNA was extracted from whole blood samples by the solid phase method [13]. The presence of the eNOS G894T polymorphism was examined by allele specific- polymerase chain reaction (ARMS-PCR) analysis [14]. Amplification by PCR of the genomic DNA of each sample includes two reactions: one with common and normal primers, and the other with common and mutant primers. In this method, two sets of primers were used for each sample: a common primer (C: 5'-ACACACACCCCTGCC- ACCCCCTACC-3') and either a normal allele-specific (N: 5'-TGCTGCTGCAGGC- CCCAGATTAG-3') or mutation-specific primer (M: 5'-TGCTGCTGCAGGCCCCA- GATTAT-3'). Amplification products were observed only in the corresponding allele-specific reactions.

PCR was performed in a volume of 20 μl, containing 30-80 ng of genomic DNA, 1×PCR buffer (with 1.5 mM of $MgCl_2$), 0.25 μM of each primer, 100 μM dNTPs and 1.5 U of Hotstart Taq DNA polymerase (Gen Star Biosolutions Co. Ltd). PCR conditions were set with an initial period of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 62 °C, 50 s at 72 °C and a final extension time of 7 min at 72 °C. The mixture of reaction was resolved by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide for analysis. Mastercycler® Gradient S thermal cycle (Eppendorf, Hamburg, Germany) PCR system were applied.

Statistical analysis

Chi-square test was used to determine whether genotype or allele frequencies of eNOS G894T in subjects was in agreement with the Hardy Weinberg equilibrium (HWE), and to compare allele frequencies of the eNOS G894T polymorphisms between patients with controls. Odds ratios (ORs) were calculated to assess the strength of association between individual genetic polymorphism and the risk of VTE. Unconditional logistic regression models were applied to calculate ORs and 95% confidence interval (CI). A p value of <0.05 was considered statistically significant. All statistical analysis was performed with the SPSS software package version 13.0.

Table 1
Basic characteristics of the VTE patients and controls.

	VTE n=462(%)	Controls n=462(%)	P
Sex			
Male	252(54.5)	253(54.8)	0.947
Female	210(45.5)	209(45.2)	
Age, y	61(17-89)	59(17-94)	0.07
BMI(kg/m2)	25.6	23.8	<0.0001
Type of VTE			
PTE	193(41.7)		
DVT	40(8.0)		
PTE+DVT	229(50.3)		
Symptom			
Dyspnea	320(69.3)		
Chest pain	104(22.7)		
Cough	91(19.7)		
Hemoptysis	28(6.1)		
Syncope	52(11.4)		
Palpitation	26(5.7)		

Results

Characteristics of the study population

The main characteristics of the patients and controls were summarized in Table 1. No significant difference in sex and age distribution was observed between the patient and healthy controls. There was a significant difference in BMI between the two groups (P<0.0001). In 462 VTE patients, there were 193 patients with isolated PE, 40 with isolated DVT and 229 with both PE and DVT.

Multivariate analysis of relative risk of VTE

Multi-variant analysis showed that the G894T polymorphism was an independent risk factor for VTE: OR 0.63, 95% CI 0.46-0.87(p=0.004) (Table 2).

The distribution of eNOS G894T polymorphism

The distribution of eNOS G894T polymorphism was in agreement with the Hardy-Weinberg equilibrium. The GT genotype frequency was significantly higher in VTE patients than in the healthy controls (18.1% vs. 12.3%, P=0.014). The frequency of GT+TT genotype was also significantly higher in VTE patients compared to controls (20.3% vs. 13.4%, P=0.005). In addition, T allele carriers were significantly higher in VTE patients compared to healthy controls (11.3% vs 7.3%, P<0.0001) (Table 3).

Association between sex and genotype distribution of eNOS gene

To examine the gender specific association with VTE, the genotype and allele frequencies of male and female cases were compared with their respective controls. The frequency of GT+TT genotype was significantly higher in the male patients in VTE group than in controls (22.2% vs. 12.6%, P=0.005). The T allele carriers were more common among the male patients in VTE group (12.5% vs control 7.1%,

Table 2
Relative risk of VTE by VTE risk factors and by G894T polymorphism of the eNOS gene.

Risk factors	P	OR(95%CI)
Age	0.018	0.99(0.98-0.99)
Sex	0.904	1.02(0.78-1.33)
BMI	<0.0001	0.93(0.89-0.97)
Smoking	<0.0001	0.56(0.42-0.76)
G894T genotype	0.004	0.63(0.46-0.87)