ABSENCE OF FACTOR V \text{ARG}^{306}\rightarrow\text{THR} AND LOW FACTOR V \text{ARG}^{306}\rightarrow\text{GLY} MUTATION PREVALENCE IN THAI BLOOD DONORS

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Abstract. Thrombosis among the Thai population is much lower than in western countries. The Thai population is protected to some extent against familial thrombophilia as by the very low prevalence of factor V Leiden, G20210A prothrombin and C677T methylenetetrahydrofolate reductase mutations. The present study reports the prevalence of two mutations of the factor V gene involving the codon for Arg 306 among 500 healthy adult voluntary blood donors (males 285, females 215) and 30 children (boys 20, girls 10) experiencing a total of 36 thrombotic episodes. The blood donors’ ages ranged from 18 to 60 years while the children’s ages ranged from 9 months to 15 years. The allelic frequencies of the factor V gene mutation of G1091C and A1090G among blood donors were 0% and 0.4%, respectively. Additionally, both mutations were not present in any of the 30 children with thrombosis. The low prevalence of factor V gene mutations in the codon Arg 306 may be relevant to the low rate of thrombosis among the Thai population.

INTRODUCTION

Human factor V acts as a cofactor in the conversion of prothrombin to thrombin in the coagulation process. It is converted to its active form factor Va, by thrombin and quickly is converted to an inactive form by activated protein C in order to maintain the hemostasis. The inactivation of factor Va begins with the initial cleavage at Arg 506 and successive cleavages at Arg 306 and Arg 679 (Kalafatis et al, 1994; 1995). The cleavage at Arg 506 is essential for the subsequent exposure of cleavage sites at Arg 306 and Arg 679.

A single amino acid substitution (Arg\textsuperscript{506}\rightarrow\text{Gln}) in the factor V molecule is caused by a single point mutation (G1691A) in the factor V gene, commonly known as factor V Leiden. This mutation is associated with an increased risk of venous thrombosis (Bertina et al, 1994; Dahlback et al, 1994; Svensson and Dahlback, 1994), commonly found in Europe (Rees, 1996; Rees et al, 1999) but rare in Asian countries (Rees et al, 1995). The absence and low prevalence (0.1%) of factor V Leiden were previously described among 500 Thai blood donors (Chuansumrit et al, 2001b) and 500 Thai healthy subjects (Angchaisuksiri et al, 2000a), respectively. Recently, a study reported the novel mutation (A1090G) resulting in a single amino acid substitution (Arg\textsuperscript{506}\rightarrow\text{Gly}) in two Hong Kong Chinese patients with thrombosis (Chan et al, 1998). Another study also reported the novel mutation (G1091C) resulting in a single amino acid substitution (Arg\textsuperscript{306}\rightarrow\text{Thr}) in patients with activated protein C resistance and thrombosis (Williamson et al, 1998).

We therefore determined the prevalence of these two mutations on the factor V gene among Thai blood donors and children with thrombosis in order to assess the importance of these genetic defects in the occurrence of thrombosis among the Thai population.

MATERIAL AND METHODS

Subjects

Five hundred regular blood donors (males 285, females 215) at the Blood Bank, Department of Pathology, Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Bangkok, were enrolled in the study from January 2002 to December 2002. In addition, 30 patients (boys 20, girls 10)
from the Department of Pediatrics experiencing thrombosis were also included in the study. The study was approved by the Faculty Ethics Committee and informed consent was obtained from the parents of the patients.

One milliliter of whole blood was mixed with EDTA as an anticoagulant and refrigerated at 4°C refrigerator. All blood donors and patients were Thai.

**Isolation of genomic DNA**

DNA was extracted from whole blood using a 5% suspension of Chelex by the following procedures (Walsh *et al.* 1991). First, 3 µl of whole blood was added to 1 ml of sterile distilled water, and incubated at room temperature for 15-30 minutes, mixed occasionally by inversion of gentle vortexing. Next, it was centrifuged in a microcentrifuge at 10,000-15,000 rpm for 2-3 minutes and the supernatant was carefully removed and discarded. Then, the 5% suspension of Chelex was added to a final volume of 200 µl and incubated at 56°C for 15-30 minutes, followed by vortexing at high speed for 2-3 seconds and placed in a boiling water bath for 8 minutes. It was then vortexed at high speed for another 5-10 seconds and spun in a microcentrifuge at 15,000 rpm for 2-3 minutes. As a result, 10 µl of the supernatant was ready for DNA amplification.

**Identification of factor V mutations at Arg^{306}**

A 200-bp DNA segment containing exon 7 of the factor V gene was amplified by PCR technique using two primers of upstream 5'-TGTCCAACCTCAGCTGGGA-3' and downstream 5'-GTATGGAACCCCAACAATCA-3' (Shen *et al.*, 2001). The reaction was as follows: 0.5 µl (100 ng) of genomic DNA was amplified in a 25 µl reaction volume consisting of Cetus buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin), 200 µM dNTPs (Pharmacia), 30 pmole of each primer and 1 unit of Taq polymerase (Perkin-Elmer). Following an initial denaturation at 94°C for 4 minutes, thermocycling of 94°C for 20 seconds, 60°C of 20 seconds and 72°C for 20 seconds was carried out for a total of 35 cycles and a final extension at 72°C for 5 minutes. The amplified product of 200 bp was obtained. Following amplification, 15 µl of products were digested with the restriction enzyme *Bst* OI (Biolabs, New England) and incubated at 60°C for 4 hours according to the manufacturer’s instructions. The *Bst* OI recognition site was CC³ (A/T) GG and two fragments of 140 bp and 60 bp were obtained from a normal sample. Both G to C transition at nucleotide 1091 (factor V Arg³⁰⁶→Thr) and A to G transition at nucleotide 1090 (factor V Arg³⁰⁶→Gly) abolished the recognition site of *Bst* OI. Then size fractionation by gel electrophoresis on 8% native acrylamide gel was performed. Samples without cleavage by *Bst* OI, to be 140 bp and 60 bp, were sequenced using an automated fluorescent sequencer. Then, the sequence chromatogram was analyzed by computer software.

**RESULTS**

All of blood donors were healthy volunteers who met the standard criteria recommended by the American Association of Blood Banks. No history of thrombosis was found. Their ages ranged from 18 to 60 years with a mean of 33 years, 2 months.

Thirty children with thrombosis, whose ages ranged from 9 months to 15 years with a mean of 8 years, 6 months also enrolled in the study. A total of 36 episodes of thrombosis involved brain (n = 21), deep veins of the leg (n = 4), lungs (n = 2), poplitical artery and vein (n = 2) and central retinal artery (n = 1). Two patients each developed two and three episodes of thrombosis, respectively. The thrombosis in the brain was diagnosed by computerized tomography or magnetic resonance imaging, deep vein thrombosis by duplex Doppler ultrasonography, pulmonary embolism by ventilation perfusion lung scan and specific vessel thrombosis by angiography. The etiologies of thrombosis were not known except in five patients who had severe protein C deficiency (n = 2), antiphospholipid syndrome (n = 2), and polycythemia (n = 1).

The factor V gene mutation of Arg³⁰⁶→Thr (G1091C) was not detected among any of the 500 Thai blood donors. The heterozygous state of the factor V gene mutation of Arg³⁰⁶→Gly (A1090G) was found in four blood donors. Therefore, the allelic frequencies of G1091C and A1090G mutations among Thai blood donors were 0% and 0.4%, respectively. However, neither factor V mutation was present in any of the 30 children with thrombosis.

**DISCUSSION**

Thrombosis among the Thai population is much lower that in western countries (Chumnijaraki and Poshyachinda, 1975; Atichartakarn *et al.*, 1988). The habitual ingestion of garlic and chilli peppers in a high fiber and low fat diet appears to be one of the contributing factors.
Most Thai are Buddhist and Buddhism teaches them to be peaceful, avoid conflict and accept adversity graciously. The geographic position of Thailand avoids the natural disasters of hurricanes, snow storms, volcanos and earthquakes. Importantly, there are abundant agricultural products such as rice and fish from the sea and river. The religion philosophy and natural resources create the positive attitude of ‘Take it easy’ ingrained in the soul of the Thais. Therefore, the less stressful environment may contribute as one of the protective factors against thrombosis among the Thais.

Apart from the habit and environment, the Thai population also appears to be protected against familial thrombophilia in the genetic context as shown by the very low prevalence of factor V Leiden (Chuansumrit et al., 2001b), G20210A prothrombin and C677T methylenetetrahydrofolate reductase mutation (Angchaisuksiri et al., 2000a,b). The present study provides additional information concerning genetically based protection against familial thrombophilia, supporting the proposition that factor V gene mutations at the codon for Arg 306 are unlikely to be associated with the occurrence of thrombosis among the Thai population. Rather, the absence of factor V gene mutations of Arg306→Thr (G1091C) and the low prevalence of Arg306→Gly (A1090G) mutations are likely to be protective factors against thrombosis in the Thai population.

In cases of children with thrombosis, investigation of the levels of protein C, protein S and antithrombin III may be useful since deficiency of these factors is more commonly found (Chuansumrit et al., 2001a), but in view of their very low frequency screening for factor V gene mutations is not likely to be cost effective among Thai children. Further comprehensive investigation of genetic predisposing factors should be continued to build a more complete picture.

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REFERENCES


