Detection of Factor V Leiden in Thai Patients with Venous Thrombosis

Wichai Prayoonwiwat, Pasra Arnutti, Motofumi Hiyoshi, Oytip Nathalang, Chamaiporn Suwanasophon, Rachapat Kokaseam, Triroj Krutvecho, Noriyuki Tatsumi

During the last 30 years, numerous genetic and acquired defects have been found in families with a notable history of venous thrombosis. These genetic defects and acquired abnormalities have been grouped into two clinically-related disorders; hereditary thrombotic disease and hereditary hypercoagulability or thrombophilia. Genetic risk factors for thrombosis include the well-known abnormalities of protein C, protein S, anti-thrombin III, fibrinogen and activated protein C resistance (APC-R). Protein C is a vitamin-K-dependent serine proteinase, which, when activated, exerts its anticoagulant effect by selectively inactivating the procoagulant factors Va and VIIIa. Resistance to APC has been shown to be a major risk factor for venous thrombosis, which has been demonstrated in 20 to 40 percent of thrombotic patients. The molecular defect underlying APC resistance has recently been identified as a G to A point mutation in the codon for arginine 506 in the factor V gene (factor V Leiden) which is a major risk factor for venous thrombosis, especially in Caucasian populations. This study is an analysis of the Thai population to determine the prevalence of the factor V Leiden mutation. Twenty-seven patients with apparent venous thrombosis were divided into two groups according to APC-R test. Thirteen patients were diagnosed as positive for n-APC-SR, ratio < 0.8 and fourteen patients were diagnosed as negative for n-APC-SR, ratio > 0.8. Two of thirteen APC-R positive patients and one of fourteen APC-R negative patients were found to have the heterozygous allele for the factor V Leiden mutation but the homozygous allele was not detected in these groups of patients. Neither the heterozygous nor homozygous Leiden mutation was detected in 200 healthy volunteer blood donors. In conclusion, our findings indicate that factor V Leiden mutation is related to venous thrombosis in Thai people. Moreover, a further study of other mutations at the activated protein C cleavage sites of factor V and factor VIII is recommended. Factor V Leiden can be detected by the loss of a recognition site for the restriction enzyme Mnl I. This mutation is common among Caucasian populations but it is rare in Japanese, Chinese and in the native populations of Africa, Australia and America. From our previous study, the heterozygous allele factor V Leiden mutation can be detected in Thai patients with venous thrombosis. The aim of this study is to determine the prevalence of the factor V Leiden mutation in the Thai population and in Thai patients with venous thrombosis.
MATERIALS AND METHODS

Subjects

Twenty-seven patients attending the Division of Hematology, Department of Medicine, Phramongkutklao Hospital, Bangkok, Thailand were included. These patients had apparent venous thrombosis and were diagnosed by APC resistance and the results were expressed in a normalized ratio (n-APC-SR). Their ages ranged from 20 to 72; the mean age was 42 years. In addition, 200 unrelated healthy volunteer blood donors without any thrombosis from the Blood Bank, Army Institute of Pathology, Bangkok, Thailand, were included in this study. Their ages ranged from 23 to 52; the mean age was 36 years. Informed consent was obtained from all subjects.

DNA Studies

Molecular analysis for the factor V Leiden mutation was performed on EDTA whole blood and the DNA was purified by the phenol-chloroform method. Polymerase chain reaction (PCR) of the exon 10 of the factor V gene was performed. The amplification solution contained 100-300 ng DNA, 100 pmol of each primer (5' sense primer: 5'-ACCCACAGAAATGATGCCCA-3'; 3' antisense primer: 5'-TGCCCATTATTAGCCAGGAG-3'). Two to four hundreds pmol of each dideoxynucleoside triphosphate, which were mixed in a total volume of 50 μl of 1 x PCR reaction buffer with 1.25 units of AmpliTaq Gold DNA polymerase (Perkin Elmer, New Jersey, USA). The reaction mixture was placed in a Perkin Elmer model 9700 thermal cycler (The Perkin Elmer Corporation, Norwalk, CT, USA) and heated for 12 minutes at 94°C and subjected to 50 cycles of amplification (91°C for 40 seconds, 55°C for 40 seconds, and 71°C for 2 minutes). Fifteen microfilters of PCR product was digested overnight with 5 units of Mnl I (New England Biolabs, MA, USA). The undigested and digested samples of PCR products were separated electrophoretically in 3% agarose gel with ethidium bromide and the bands were visualized using a UV transilluminator. The undigested PCR product showed 223 base pairs (bp) and after being cleaved with Mnl I, a normal allele produced bands of 37, 82 and 104 bp, while the factor V Leiden allele produced the homozygous pattern bands of 82 and 141 bp. Moreover, bands 37, 82, 104 and 141 were produced by the heterozygous allele.

RESULTS

Twenty-seven patients with apparent venous thrombosis were divided into two groups according to the APC-R test. Thirteen patients were diagnosed as positive for n-APC-SR, ratio < 0.8 and fourteen patients were diagnosed as negative for n-APC-SR, ratio > 0.8. Two of thirteen APC-R positive patients and one of fourteen-APC-R negative patients were found to have the heterozygous allele for the factor V Leiden mutation (Fig 1) but the homozygous allele was not detected in these groups of patients. Neither the heterozygous nor homozygous Leiden mutation was detected in 200 healthy volunteers.

DISCUSSION

This study was undertaken to determine the prevalence of the factor V Leiden mutation in the Thai population and in Thai patients with venous thrombosis. This mutation is most common in Caucasian populations but very few cases have been found in Asia. Currently, research supports the theory that the Leiden mutation occurred after Homo sapiens separated into Caucasian and Mongolian populations. Five cases of the Leiden mutation have been detected in Indian and Polynesian populations, which are likely to be the result of admixture with colonizing Europeans. Moreover, recent data have discovered the heterozygous allele factor V Leiden in two Thai thrombosis patients with APC-R positive test results. The APC-R test is a screening test and a positive result is usually linked to factor V Leiden, but may occur in other disorders associated with hypercoagulability, high factor VIII levels, lupus anticoagulant and other acquired conditions such as pregnancy and oral contraceptive drug use.

In a study of 13 thrombosis patients with APC-R positive results, the heterozygous factor V Leiden was found in two patients and one patient was found with heterozygous factor V Leiden with APC-R negative results. Thus, even when the heterozygous factor V Leiden with APC-R negative results can be detected in patients; it is suggested that the molecular analysis for factor V Leiden be done to explain the cause of thrombosis. Therefore, our findings indicate that factor V Leiden mutation is related to venous thrombosis in Thai people. Moreover, a further study of other mutations at the activated protein C cleavage sites of factor V and factor VIII is recommended.
FACTOR V LEIDEN IN THAI PATIENTS

Fig. 1 Anticoagulant response to APC; right = 3 patients heterozygous for Arg506→Gln mutation, Left = 24 patients without the mutation.

REFERENCES


