

Original Article

Prevalence of Factor V Leiden (G1691A) and Prothrombin Gene Mutation (G20210A) Among Different Ethnic Groups in Thai Hospitals

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Abstract: Factor V Leiden and prothrombin gene mutation are the most common genetic risk factors of venous thromboembolism (VTE) in Western countries. We evaluated the prevalence of these two mutations from blood samples of patients with clinically suspected thromboembolic disease sent to our laboratory. **Methods:** During January 2002 to March 2010, 797 blood samples from the government and private hospitals were assayed for the mutations by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. **Results:** The number of samples from government hospitals was 208, comprising 200 Thais, 1 Asian, 2 Middle-Easterners and 5 Caucasians; whereas those from private hospitals were 589, comprising 169 Thais, 49 Asians, 112 Middle-Easterners, and 259 Caucasians. The prevalence of homozygosity and heterozygosity for factor V Leiden was 0.1% and 6.5%, respectively. The prevalence of heterozygosity for prothrombin gene mutation was 1.9%. One each of Caucasian origin was a compound heterozygote for both genes, and a combined homozygote for factor V Leiden and heterozygote for prothrombin gene mutation, respectively. Factor V Leiden was more frequently found in male than in female (9.9% vs. 2.8%, $p < 0.001$) but no gender difference was found in the prothrombin gene mutation. The frequency of factor V Leiden was significantly higher among Caucasians compared to Middle-Easterners, other Asians, and Thais (16.7% vs. 4.6%, 8.0%, and 0%, respectively, $p < 0.001$). Higher frequency was also seen in prothrombin gene mutation among Caucasians compared to the other three groups (3.8% vs. 2.4%, 0%, and 0.7%, respectively, $p = 0.05$). **Conclusion:** The results from our laboratory confirmed the higher frequency of these two mutations among Caucasians than other ethnic groups as previously reported.

Key Words : ● Factor V Leiden ● Prothrombin gene mutation ● Ethnic groups

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Introduction

Venous thromboembolism (VTE) is a serious and life-threatening condition resulting from a combination of genetic and acquired or environmental factors. The most common genetic defect for hereditary thrombophilia in Western countries is factor V Leiden. In the coagulation cascade, activated factor V functions as a co-factor in

the activation of prothrombin by activated factor X. Thrombin converts fibrinogen to fibrin that polymerizes to form a clot. Activated protein C (aPC) is a natural anticoagulant that acts to limit the extent of clotting by cleaving and degrading factor Va and factor VIIIa. The mutation of factor V is due to the substitution of guanine (G) to adenine (A) at nucleotide 1,691 of gene coding for factor V that results in the changing of amino acid from arginine (CGA) to glutamine (CAA) at amino acid position 506.^{1,2} Since this amino acid is the cleavage site for aPC, the mutation results in a factor V variant, which cannot be easily degraded by

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aPC.³⁻⁴ When factor Va remains active, thrombin is overproduced leading to excess fibrin generation and clot formation. Heterozygote and homozygote of factor V Leiden are associated with a 7- and 80-fold increased risk of venous thrombosis, respectively.⁵ Factor V Leiden is common among Caucasians and Middle-Easterners with the prevalence ranging from 2% to 9.5% and 2% to 13.8%, respectively. Moderate prevalence is found in Hispanic Americans and Indians (1-5%), whereas it is rare in other Asians and Africans (< 1%).⁶⁻¹⁶

The second most common genetic risk factor for hereditary thrombophilia is the prothrombin gene mutation, which is due to the substitution of G to A at nucleotide position 20210 of the prothrombin gene leading to an increased prothrombin production. This defect is associated with an increased risk of venous thrombosis by three folds.¹⁷ Its prevalence among Caucasians and Middle-Easterners is approximately 1% to 5.7% but <1% among Asians and Africans.^{8-10,13-16,18}

We hereby report the prevalence of factor V Leiden and prothrombin G20210A mutations in blood samples sent to our laboratory as part of the evaluation of patients with clinically suspected thromboembolic diseases.

Materials and Methods

Blood samples

Blood samples of 797 patients with clinically suspected thromboembolic disease from government and private hospitals collected in vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) during January 2002 to March 2010 were assayed for factor V Leiden and/or prothrombin G20210A mutation. Patients' ages ranged from 2 to 94 years with a male to female ratio of 1 to 1.15.

Methods

Extraction of DNA

Genomic DNA was obtained from peripheral blood leucocytes using sodium dodecyl sulphate (SDS) as cell lysing and proteinase K as a protein digesting reagent. DNA was extracted by phenol/chloroform and

was precipitated in ethanol.¹⁹

Identification of factor V Leiden

Factor V Leiden was determined by the polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) and followed by *Hind* III digestion.²⁰ PCR reactions were performed according to standardized procedures on Thermal Cycler (Bio- Rad; Hercules, CA, USA). The PCR products were subjected to restriction enzyme (*Hind* III) digestion overnight at 37° C. The products were analyzed on 3% Nusieve agarose gel electrophoresis and the separated DNA bands were visualized by ethidium bromide.

When the subject was homozygous for the normal codon corresponding to amino acid 506 (Arg/Arg), there was no restriction site for *Hind*III, and the fragment remained undigested (241 bp). When the subject was homozygous for the codon corresponding to Gln at position 506 (Gln/Gln), a restriction site for *Hind*III was created allowing the fragment to be completely digested into two fragments of 209 and 32 bp (the latter was not visible on the gel). When the subject was heterozygous, both patterns were visible corresponding to the undigested (241 bp) and digested (209 bp) amplified fragments.

Identification of prothrombin G20210A gene mutation

The detection of prothrombin gene mutation was performed according to the method described by Poort SR et al.¹⁷ The undigested fragment of normal subject was 345 bp whereas digested fragments of homozygous mutation were 322 and 23 bp (invisible on the gel). The heterozygote contained two fragments of 354 and 322 bp.

Statistical analysis

The statistical analysis was performed by SPSS 11.5 for Windows. Prevalence of factor V Leiden and prothrombin gene mutation were determined from the genotype frequencies. Comparisons between groups was calculated by the Chi-square test.

Results

The number of samples from government hospitals were 208, comprising 200 Thais, 1 other Asian, 2 Middle-

Easterners, and 5 Caucasians, whereas those from private hospitals were 589, comprising 169 Thais, 49 other Asians, 112 Middle-Easterners, and 259 Caucasians. The proportion of male to female among Thais, other Asians, Middle-Easterners, and Caucasians were 1:1.5, 2.3:1, 1.1:1, and 2.3:1, respectively.

The prevalence of heterozygous, and homozygous factor V Leiden was 6.5%, and 0.1%, respectively. For prothrombin gene mutation, the prevalence of heterozygote, and homozygote was 1.9%, and 0%, respectively. One Caucasian patient had a combination of both heterozygous genes. The other was homozygous for factor V Leiden and heterozygous for prothrombin gene mutation.

The prevalence of factor V Leiden and prothrombin gene mutation according to age range was shown in

Table 1. No factor V Leiden was detected at age range above 80 years and no prothrombin gene mutation was detected at age range above 60 years. Factor V Leiden was more frequently found in male than in female (9.9% vs. 2.8%, $p < 0.001$). No gender difference was found in prothrombin gene mutation (2.1% vs. 1.7%, $p = 0.47$).

The frequency of factor V Leiden was significantly higher among Caucasians compared to Middle-Easterners, other Asians, and Thais (16.7%, 4.6%, 8.0% and 0%, respectively, $p < 0.001$). Higher frequency was also seen in prothrombin gene mutation among Caucasians compared to the other three groups (3.8%, 2.4%, 0%, and 0.7%, respectively, $p = 0.05$) (Table 2).

Table 1. Frequency of factor V Leiden and prothrombin gene mutation according to age range.

Age range (years)	Factor V Leiden Number (%)	Prothrombin gene mutation Number (%)
0-20	4/45 (8.9)	1/30 (3.3)
21-40	18/215 (8.4)	4/179 (2.2)
41-60	15/249 (6.0)	5/206 (2.4)
61-80	7/82 (8.5)	0/67 (0.0)
81-100	0/14 (0.0)	0/12 (0.0)

Table 2. Frequency of factor V Leiden and prothrombin gene mutation among different ethnic groups according to sex.

Ethnic groups	Factor V Leiden n/N (%)	Prothrombin gene mutation n/N (%)
Caucasian	42/252 (16.7)	8/208 (3.8)
Male	34/175 (19.4)	6/145 (4.1)
Female	8/77 (10.4)	2/63 (3.2)
Middle-easterner	5/109 (4.6)	2/83 (2.4)
Male	3/57 (5.3)	1/44 (2.3)
Female	2/52 (3.8)	1/39 (2.6)
Other Asians	4/50 (8.0)	0/39 (0)
Male	4/35 (11.4)	0/29 (0)
Female	0/15 (0)	0/10 (0)
Thai	0/358 (0)	2/295 (0.7)
Male	0/144 (0)	0/114 (0)
Female	0/214 (0)	2/181 (1.1)

Discussion

We studied the prevalence of factor V Leiden and prothrombin gene mutations in patients with thromboembolism in different ethnic groups. The prevalence of homozygosity and heterozygosity for factor V Leiden was 0.1% and 6.5%, respectively. The prevalence of heterozygosity for prothrombin gene mutation was 1.9%.

There was a gender difference in the prevalence of factor V Leiden, with male being significantly higher than female (9.9% vs. 2.8%). However, the gender difference was not seen in prothrombin gene mutation. The higher prevalence of factor V Leiden in male has not been previously reported. This finding needs to be confirmed in the larger number of samples. There may be some selective advantages for the carrier status such as reduced bleeding after trauma, particularly in times when hunting-associated trauma was more common and medical care was highly primitive.

Frequencies of factor V Leiden and prothrombin gene mutation were significantly higher in Caucasians than those in other ethnic groups as previously reported. Friedline JA et al.²¹ and Wysokinska EM et al.²² from the USA reported the prevalence of factor V Leiden and prothrombin gene mutation in study groups of 401 and 163 Caucasian patients presenting with thromboembolic diseases, respectively. The first group of the 401 patients had 8% heterozygous and 1% homozygous factor V Leiden and 5% heterozygous prothrombin gene mutation. The combination of both genes was 0.5%. The second group of the 163 patients had only heterozygous of factor V Leiden and prothrombin gene mutation (10% and 11%,

respectively). High prevalence of the two defects in Caucasians was also confirmed by the reports from Europe. The study groups were 366, 149, and 119 patients with venous thromboembolism (VTE) and 51 patients with pulmonary embolism from France, Poland, Greece and Bulgaria, respectively. The prevalence of factor V Leiden ranged from 12.5% to 23.5% and of prothrombin gene mutation from 4% to 10%.²³⁻²⁶ A study from Israel²⁷ showed a high prevalence of factor V Leiden and prothrombin gene mutation at 40.1% and 18.5%, respectively in 162 thrombotic patients. The results from Jordan²⁸ and Iran²⁹ for factor V Leiden were 23.9% and 11.4% in 92 acute pulmonary embolism and 80 deep vein thrombosis patients, respectively, and those of prothrombin gene mutation were 3.3% and 3.8%, respectively. Only factor V Leiden was significantly higher than control groups. On the other hand, the frequencies of these genes in 64 Brazilian cancer patients with venous thrombosis were rather low with the prevalence as 1.5% for both mutations.³⁰ Angchaisuksiri et al.¹⁵ did not find any carrier of these genes in 50 Thai VTE patients. The prevalence of factor V Leiden and prothrombin gene mutation has also been shown to be rare or absent in the Thai population from other studies.³¹⁻³³ The prevalence of factor V Leiden and prothrombin gene mutation in other Asian countries was summarized in Table 3.³⁴

Our findings confirmed that the prevalence of factor V Leiden and prothrombin gene mutation was higher among Caucasians than in other ethnic groups. Factor V Leiden and prothrombin gene mutation are not common among Thais. Therefore, screening for factor V Leiden

Table 3. Frequency of factor V Leiden and prothrombin gene mutation among patients with Venous thromboembolism (VTE) in different ethnic groups.³⁴

	Prevalence in Western patients with VTE (%)	Prevalence in Thai patients with VTE (%)	Prevalence in Chinese patients with VTE (%)	Prevalence in Japanese patients with VTE (%)	Prevalence in Indian patients with VTE (%)
Factor V Leiden	18.8	0	0	0	3
Prothrombin gene mutation	7.1	0	0	0	0

and prothrombin gene mutation is of limited benefit and is not cost effective in Thai patients.

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ความชุกของ Factor V Leiden (G1691A) และ Prothrombin Gene Mutation (G20210A) ในกลุ่มคนที่มีเชื้อชาติแตกต่างกันในโรงพยาบาลของไทย

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หน่วยโลหิตวิทยา ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

บทคัดย่อ: การกลายพันธุ์ของ factor V ที่เรียกว่า factor V Leiden และการกลายพันธุ์ของ prothrombin gene ถือเป็นปัจจัยเสี่ยงทางด้านพันธุกรรมที่พบได้บ่อยที่สุดของการเกิดภาวะหลอดเลือดดำอุดตัน คณะผู้วิจัยได้ประเมินความชุกของการกลายพันธุ์ทั้งสองจากตัวอย่างเลือดของผู้ป่วยที่มีภาวะหลอดเลือดดำอุดตันที่ส่งมาตรวจหาปัจจัยทั้งสองยังห้องปฏิบัติการของหน่วยโลหิตวิทยา **วิธีการศึกษา:** ระหว่างมกราคม 2545 ถึงมีนาคม 2553 ตัวอย่างเลือดจำนวน 797 รายที่ส่งมาจากโรงพยาบาลรัฐบาลและโรงพยาบาลเอกชนได้ถูกวิเคราะห์เพื่อหาการกลายพันธุ์ทั้งสองโดยวิธี polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) **ผลการทดลอง:** จำนวนตัวอย่างเลือดที่ส่งมาจากโรงพยาบาลรัฐบาลมีจำนวน 208 รายประกอบด้วยคนไทย 200 ราย คนเอเชีย 2 ราย คนตะวันออกกลาง 2 รายและคนคอเคเซียน 5 ราย ที่มาจากโรงพยาบาลเอกชนมี 589 รายประกอบด้วยคนไทย 169 ราย คนเอเชีย 49 ราย คนตะวันออกกลาง 112 รายและคนคอเคเซียน 259 ราย พบความชุกของ factor V Leiden ชนิด homozygous ร้อยละ 0.1 ชนิด heterozygous ร้อยละ 6.5 ส่วนความชุกของ prothrombin gene mutation พบชนิด heterozygous ร้อยละ 1.9 คนคอเคเซียนคนหนึ่งเป็น heterozygote ของทั้งสองยีนและอีกคนหนึ่งเป็น homozygote ของ factor V Leiden และ heterozygote ของ prothrombin gene mutation เพศชายจะพบความถี่ของ factor V Leiden บ่อยกว่าเพศหญิง (ร้อยละ 9.9 เทียบกับร้อยละ 2.8, $p < 0.001$) ส่วน prothrombin gene mutation ไม่พบความแตกต่างระหว่างเพศ ความถี่ของ factor V Leiden ในคนคอเคเซียนพบว่าสูงกว่าในคนจากตะวันออกกลาง คนเอเชียและคนไทย อย่างมีนัยสำคัญทางสถิติ (ร้อยละ 16.7 เทียบกับร้อยละ 4.6 ร้อยละ 8.0 และร้อยละ 0 ตามลำดับ $p < 0.001$) ส่วนความถี่ของ prothrombin gene mutation ก็พบว่าในคนคอเคเซียนสูงกว่าคนอื่นอีก 3 เชื้อชาติเช่นกัน (ร้อยละ 3.8 เทียบกับร้อยละ 2.4 ร้อยละ 0 และร้อยละ 0.7 ตามลำดับ $p = 0.05$) **สรุป:** ผลการการศึกษานี้ยืนยันว่าความถี่ของการกลายพันธุ์ของยีนทั้งสองพบได้สูงในคนคอเคเซียนมากกว่าคนจากเชื้อชาติอื่นดังที่ได้มีการรายงานไว้แล้ว

Key Words: ● Factor V Leiden ● Prothrombin gene mutation ● เชื้อชาติ

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2555;22:115-20.