

## Association of factor V Leiden mutation with deep vein thrombosis among Egyptian cases

\*Sherif H. Abdeen, \*\*Rizk El-Baz, \*Wessam El-Gazar, \*\*Ahmad Settin

\*Zoology Department, Faculty of Science, Mansoura University

\*\*Genetics Unit, Faculty of Medicine, Mansoura University

### Abstract

**Background:** Deep vein thrombosis (DVT) is a blood clot in a major vein, usually in the legs and/or pelvis. If part of the thrombus breaks off, it becomes an embolism, which can travel through the heart and block the arteries to the lungs. Factor V Leiden (FVL) is a common genetic risk factor for hereditary hypercoagulability disorder in several populations. The present study investigates the association of FVL mutation with DVT among Egyptian cases.

**Patients & methods:** The study included 44 cases (16 males and 28 females) with an age range of 20 to 80 years in addition to 211 healthy unrelated controls of matched age and sex. A multiplex allele-specific PCR amplification was conducted for assignment of FVL gene mutation (G1691A).

**Results:** Cases having the mutant allele A (AA and AG genotypes) were significantly higher than controls (38.6% vs. 18.5%;  $P < 0.05$ , OR= 2.78 and CI 95%, 1.380–5.589).

**Conclusion:** These results concluded that FVL mutation has a high frequency and positive association with the occurrence of deep vein thrombosis among Egyptian cases.

Keywords: factor V Leiden mutation, deep vein thrombosis, Egyptian population

### Introduction:

Deep vein thrombosis (DVT) refers to a thrombus in the deep veins, most often in the lower extremities. The thrombus can embolize and become lodged in the pulmonary arteries, resulting in pulmonary embolism (PE). Deep venous thrombosis is a common disease with genetic and acquired risk factors. Acquired conditions that promote DVT include prolonged immobilization, major injuries and surgery. Hereditary coagulation defects associated with an increased risk of DVT are protein C, protein S, anti-thrombin deficiency, factor II (20210 G to A) mutation, activated protein C resistance, and factor V Leiden (FVL) mutation (Lane *et al.*, 1996; Eekhoff *et al.*, 2000; Michota, 2005).

Coagulation factor V (FV) is an important procoagulant protein, its activated form (FVa) is functioning as a cofactor in the generation of thrombin (Stormorken, 2003). FV is a single-chain mosaic domain structure composed of three

homologous A-type domains, two smaller C-type domains and a large B-domain (A1-A2-B-A3-C1-C2) (Nesheim *et al.*, 1979; Jenny *et al.*, 1987). Proteolytic activation of FV results in the removal of the FV B-domain and the exposure of regions in the FV molecule that is important for the expression of its procoagulant activity (Steen and Dahlbak, 2002; Toso and Camire, 2004). The released FVa is composed of a 105 kDa heavy chain (A1-A2 domains) and a 74 to 71 kDa light chain (A3-C1-C2 domains), held together by a single calcium ion and hydrophobic interactions (Nicolaes and Dahlback, 2002; Mann and Kalafatis, 2003). Besides circulating in free form in plasma, FV is also present in the granules of the platelets; this form accounts for about 25% of the total FV content in human blood (Chesney *et al.*, 1981). During coagulation, platelet FV is secreted as a result of platelet activation. Although several cellular types

have been reported to synthesize FV, it is generally accepted that the principal site of its biosynthesis is the liver, where human FV is synthesized as a single-chain molecule, undergoing extensive post-translational modifications before being secreted into the blood (Owen and Bowie, 1977; Wilson *et al.*, 1984).

The corresponding FV gene map on chromosome 1q23 is composed by 25 exons, spanning a chromosomal region of about 80 kb. The encoded 2224 amino-acid pre-cofactor includes a 28-residues signal peptide (Nicolaes and Dahlback, 2002; Mann and Kalafatis, 2003; Duga *et al.*, 2004). FV Leiden is currently the most common known genetic risk factor for the inherited thrombophilia. The factor V gene defect occurs in exon 10 where there is a G-A substitution at nucleotide 1691 (Bertina *et al.*, 1994). This mutation renders the factor V protein resistant to proteolytic inactivation by activated protein C and thus predisposes to thrombosis (Svensson and Dahlback, 1994).

The function of protein C is to inactivate factor Va and factor VIIIa (the 'a' denotes the active form). The first step in this process is the activation of thrombomodulin by thrombin. Subsequently, protein C combines with thrombomodulin in order to produce activated Protein C (Eisenberg *et al.*, 1993). Activated protein C then combines with protein S on the surface of the platelets. Activated protein C can then degrade factor Va and factor VIIIa. When one has factor V Leiden, the factor Va is resistant to the normal effects of activated protein C, thus the term activated protein C resistance. The result is that FVL is inactivated by activated protein C at a much slower rate, thus leading to a thrombophilic (propensity to clot) state by having increased activity of factor V in the blood (Hobikoglu *et al.*, 2004).

Heterozygotes for FVL have an approximate eightfold increased relative risk for the development of venous thrombosis and homozygotes are estimated to have an approximately 90-fold increased

relative risk (Bertina, 1997). The mutation is also a risk factor for cerebral, mesenteric and portal vein thrombosis. There is evidence that FVL mutation, presumably due to thrombosis of placental vessels, may play a role in some cases of unexplained recurrent pregnancy loss (Preston *et al.*, 1996; Ridker *et al.*, 1998; Tormene *et al.*, 1999; Martinelli *et al.*, 2000; Stolz *et al.*, 2000; Agaoglu *et al.*, 2003; El-Karakasy *et al.*, 2004).

From all of the previously mentioned literatures, it was therefore of interest to carry out experiments investigating the existence of FVL gene mutations and its association with hereditary thrombophilia among Egyptians.

### Patients and Methods:

The patients included forty four (16 males and 28 females) suffering deep vein thrombosis (DVT), confirmed by ultrasonography. All clinical data are available at the Department of Surgery, Internal Medicine, Mansoura University Hospitals, Egypt. FVL genotypes and allele frequencies were compared to 211 healthy unrelated control cases of matched age and sex from the same locality. After obtaining informed consent from all cases and controls, 3 ml venous blood sample were collected from each subject for DNA extraction and purification (Gentra systems, USA). FVL gene mutation (G1691A) was detected using a multiplex allele-specific PCR amplification for both FVL gene and factor IX as an internal control. Each PCR was performed with 300 ng of DNA, 200 mmol/L of each dNTP, 500 mmol/L of each primer, and 2.5 units of Taq DNA polymerase (Amplitaq Gold, Perkin-Elmer Cetus, Norwalk, Conn). DNA was initially denatured for 10 minutes at 95°C, and then 10 cycles were performed as follows: 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute. Then, 25 cycles were performed as follows: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. The PCR amplification was completed by a final extension at 72°C for 7 minutes. Amplification of the factor V gene

resulted in 152 base pair (bp) product while the internal amplification control (factor IX gene) resulted in a 250 bp product. The amplified products were then electrophoresed in 2% agarose gel, stained with ethidium bromide, and visualized under UV light. The primers were designed following Hezard *et al.* (1998) and Ranguelov *et al.* (2002) and their sequences were as follows:

FV common 5'- GGA CTA CTT GAC AATTAC TGT TCT CTT G 3'.

FV WT (wild type) 5'- GCA GAT CCC TGG ACA GAC G 3'.

FV MT (mutant type) 5'- GCA GAT CCC TGG ACA GAC A 3'.

FIX-1 5'- CTC CTG CAG CAT TGA GGGAGA TGG ACA TT 3'.

FIX-2 5'- CTC GAA TTCGGC AAG CAT ACT CAA TGT AT 3'

### Statistical analysis

Data were analyzed using SPSS statistical package software for calculation of genotype and allele frequencies. Testing association and risk related to FVL and DVT was done by comparing genotype and allele frequencies in cases and controls using Fisher's exact test for continuity correction together with odds ratio (OR) and 95% confidence intervals. Moreover, Hardy-Weinberg test was applied on genotype and allele frequencies of controls to check for significance of difference between observed and expected genotype frequencies and found non-significant using Chi Square test.

### Results:

The present study included 44 patients suffering DVT. The patients included 16 (36.4%) males and 28 (63.6%) females, all in the age range of 20-80 years. Another 211 healthy individuals, with no history of any vein thrombosis diseases, were also included as controls. The age and sex of the control group were approximately similar to those of the patients.

The total cases showed non-significant frequency of the FVL heterozygous mutant genotype GA among

DVT cases (27.3%) compared with that of the controls (16.6%), ( $P > 0.05$ , OR=1.886 and CI (95%, 0.8851–4.017). The total cases showed a significant higher frequency of the homozygous mutant genotype AA among the patients group (11.4%) compared with that of the controls (1.9%), ( $P < 0.01$ , OR= 6.635 and CI (95%, 1.705–25.821).

Therefore, the total cases showed a highly significant frequency of the total FVL mutant genotypes AA and GA (homozygous & heterozygous, respectively) among DVT cases (38.6%) compared with that of the controls (18.5%), ( $P < 0.0001$ , OR= 2.777 and CI (95%, 1.380–5.589). Regarding allelic frequencies, the mutant A allele was in 22(25%) cases and 43(10.19%) controls ( $P < 0.01$ , OR= 2.938 and CI (95%, 1.651-5.229). On the other hand, the normal G allele was in 66 (75%) cases and 379 (89.81%) controls ( $P < 0.01$ , OR= 0.3404 and CI (95%, 0.1912-0.6058; Table 1, Fig. 1).

### Discussion:

The Leiden mutation of blood coagulation factor V could be considered the commonest genetic abnormality associated with venous thromboembolism (Bertina *et al.*, 1994). There are several reports elaborating the fact that Factor V Leiden is a well established risk factor for deep vein thrombosis in several populations (Folsom *et al.*, 2002; Bouaziz-Borgi *et al.*, 2006; Biswas *et al.*, 2008).

In Tunisian population, it was found that FVL genotypes were present at higher frequencies in DVT patients, and increased prevalence of single mutant (1691A/4070G and 1691G/4070A), but not double mutant (1691A/4070A) haplotypes were seen among DVT patients (Bouaziz-Borgi *et al.*, 2007).

In USA, a study based on 4047 American men and women found that a 12% incidence of heterozygosity for the FVL mutation with DVT or pulmonary embolism compared with 6% in controls (Ridker *et al.*, 1997). In Switzerland, another study showed that FVL heterozygotes had a nearly eightfold lower

incidence of DVT involving the iliofemoral veins and significantly fewer extensive thromboses compared to individuals without the mutation (de Moerloose *et al.*, 2000). In Germanium population isolated DVT was also the most common major thrombotic event in a large cohort of FVL homozygotes (Ehrenforth *et al.*, 2004). In Italian population several earlier studies were suggested that individuals heterozygous for FVL had a two- to fourfold increased risk of recurrent thrombosis (Simioni *et al.*, 1997; 2000), although other studies in Austrian, Italian and Sweden population found no significant increase in risk (Eichinger *et al.*, 1997; De Stefano *et al.*, 1999; Lindmarker *et al.*, 1999).

The present study showed a significant higher frequency of the homozygous mutant genotype AA among the patient group (11.4%) compared with that of the controls (1.9%), (OR= 6.635,  $P < 0.05$ ). The total cases showed non significant higher frequency of the factor V Leiden heterozygous mutant genotype GA among DVT cases (27.3%) compared with that of the controls (16.6%), (OR=1.886,  $P > 0.05$ ). Combining both heterozygous and homozygous genotype frequency of FVL mutation i.e. all allele A carriers (AA+GA), cases showed an extremely significant higher frequency of both genotypes compared with that of the controls (38.6 % vs. 18.5%, OR= 2.777,  $P < 0.0001$ ). Regarding allelic frequencies, the mutant A allele was 22 (25%) in cases and 43 (10.19%) in controls (OR= 2.938,  $P < 0.01$ ). On the other hand, the normal G allele was 66 (75%) in cases and 379 (89.81%) in controls (OR= 0.3404,  $P < 0.01$ ).

These results may indicate that thrombophilia due to the presence of the FVL mutation is relatively high in Egyptian DVT patients as well. Moreover, there was no statistical significant difference between cases subgroups such as cases with age  $> 50$  years vs. cases  $< 50$  years, male cases vs. female cases, anemic cases vs. nonanemic cases, high oedema vs. cases low oedema, high platelet cases vs. low platelet and cases with high prothrombin ratio vs. with low prothrombin ratio, all

with positivity for mutant FVL mutation (GA + AA).

Interestingly, frequencies of FVL mutation among Egyptian deep vein thrombosis cases and controls were higher than that reported in other Mediterranean countries as well as Western countries as evidenced from the later-mentioned studies.

In Kurdish population, it has been reported that the prevalence of FVL among healthy individuals of Kurdish ethnic background in Western Iran. Factor V G1691A mutation was detected as heterozygous in 11 of 404 healthy individuals (five female and six male) and as homozygous in one male indicating a prevalence of 2.97% (95% CI, 1.3-4.6) and allele frequency of 1.6%. This indicated that the FVL is not rare among populations of Western Iran (Rahimi *et al.*, 2008).

Also in Asian-Indian population, it has been confirmed that FVL mutation was significantly associated with the risk of DVT. FVL was seen in 16 out of 155 patients (10.3%). Thirty-one of patients showed activated protein C resistance of which only 16 carried FVL mutation which was a far lower number than what is usually seen in Caucasian population. Of the 16 patients, 4 (2.58%) were homozygous for the mutant type and the rest i.e., 12 (7.72%) were heterozygous. Only one of the healthy controls was heterozygous for FVL ( $P < 0.01$ , 95% CI; OR: 13.7; Biswas *et al.*, 2008).

In Netherlands Dutch population, there was a high prevalence of inherited thrombophilia as manifested by the presence of FVL with few acquired risk factors for thrombosis. It has been found that the synergy index between minor events (short periods of immobilization such as prolonged travel, short illness, minor surgery or injuries) and FVL mutation in the case-only analysis was 0.7 (95% CI, OD: 0.3-1.5). Therefore, persons with FVL mutation who experience a minor event will have an estimated risk increase of about 17-fold, which exceeds the sum of the individual risk factors (Eekhoff *et al.*, 2000).

In Macedonian population, it has been found that FVL carriers have the highest odds of developing DVT; 21.1%

(95% CI, 14.3-25.7), compared with 5.5% in the healthy individuals (Arsov *et al.*, 2006).

In Arab communities, it has been found that FVL was a common genetic risk factor for DVT in both communities Lebanon and Tunisia. Subjects comprised 198 DVT patients and 540 healthy controls from Lebanon and 126 Tunisian DVT patients and 197 control subjects; FVL (MnlI) genotyping was done by PCR-RFLP. The prevalence of FVL mutant A allele and the G/A and A/A genotypes were significantly higher among DVT patients from Lebanon and Tunisia (Bouaziz-Borgiet *al.*, 2007).

In American population, it has been reported that the incidence of abnormality in patients with DVT was 27/44 (61%; 95% CI 47–76%) and 10 of these patients were positive for FVL (23%; 95% CI, 10–35%; Caprini *et al.*, 2005).

On the other hand, in the Chinese population it has been shown that FVL is very rare, through study on 178 patients with DVT and 102 control subjects (Jun *et al.*, 2006).

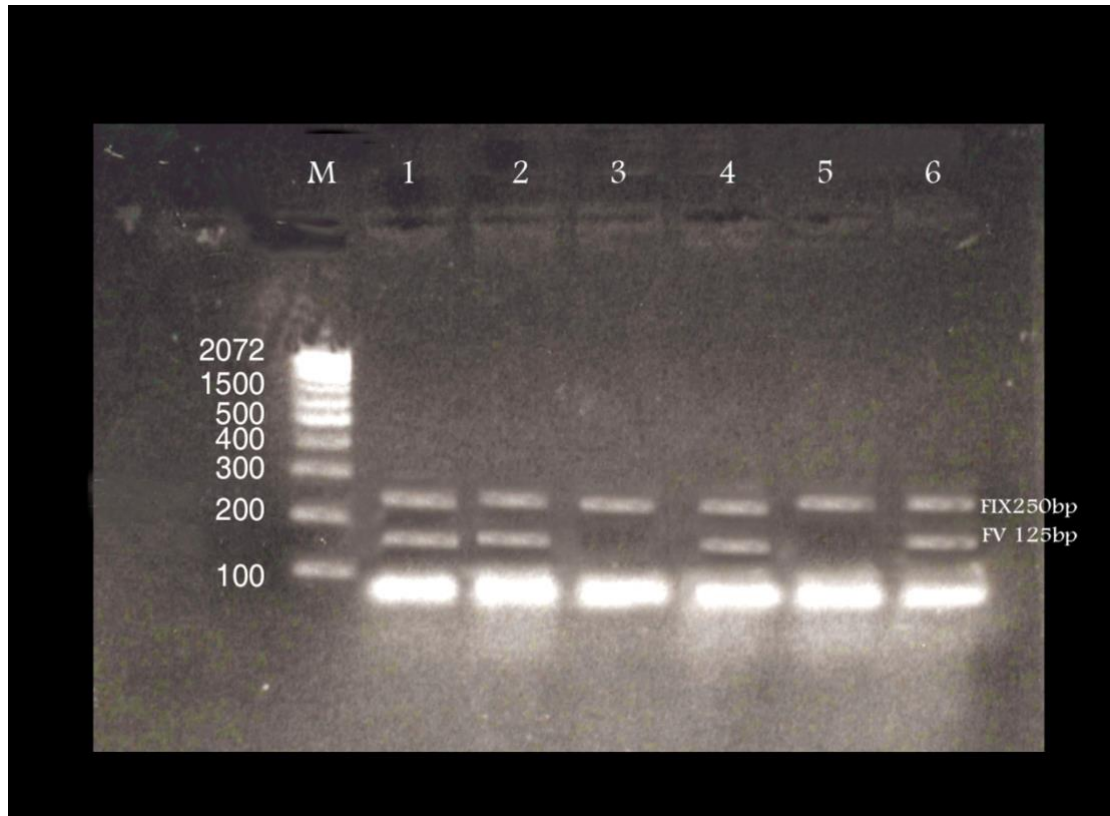
In conclusion, the present study may help for better understanding for the hereditary causes of thrombophilia in Egyptian population.

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**Figure 1.** Amplification of factor V Leiden (FVL) using primers for mutant A alleles (lanes 1, 3, 5) and normal G allele (lanes 2, 4, 6) showing positive bands (125 bp) in lane 1, 2, 4, 6 with negative bands in lanes 3, 5 indicating G/A heterozygous genotype in case 1 (lanes 1, 2) and G/G normal genotype in cases 2, 3 (lanes 3, 4, 5, 6). M indicates molecular marker, Bands of size 250 bp belongs to FIX used as internal controls.



**Table 1.** Frequency of factor V Leiden mutation among deep vein thrombosis cases compared to controls.

<b>Genotype</b>	<b>Total Cases N (%)</b>	<b>Control N (%)</b>	<b>P-value</b>	<b>OR (95% CI)</b>
<b>Individual genotype frequency</b>				
<b>Total</b>	44 (100)	211(100)		
<b>GG</b>	27 (61.3 %)	172 (81.5)	P < 0.01 **	0.360 (0.1789-0.7248)
<b>GA</b>	12 (27.3%)	35 (16.6)	P > 0.05	1.89 (0.8851-4.017)
<b>AA</b>	5 (11.4%)	4 (1.9)	P < 0.01 **	6.64 (1.705-25.821)
<b>Total homozygous mutant &amp; heterozygous</b>				
<b>AA + GA</b>	17/44 (38.6%)	39 (18.5)	P < 0.01 **	2.78 (1.380-5.589)
<b>Individual allele frequency</b>				
<b>A</b>	22/88 (25 %)	43 (10.19)	P < 0.001 ***	2.94 (1.651-5.229)
<b>G</b>	66/88 (75%)	379 (89.81)	P < 0.001 ***	0.34 (0.1912-0.6058)

\*\*P < 0.01 highly significant

\*\*\*P < 0.001 very highly significant

P: probability, OR: odds ratio, CI: confidence intervals and N = number of cases

GG homozygous wild type, GA heterozygous, AA homozygous mutant



## ارتباط طفرة العامل الخامس لايدن بالانسداد الوريدي العميق بين حالات مصرية

\*شريف حلمى عابدين ، \*\*رزق الباز ، \*وسام الجزار ، \*\*أحمد ستين  
\*قسم علم الحيوان - كلية العلوم - جامعة المنصورة  
\*\*وحدة الوراثة - كلية الطب - جامعة المنصورة

**الخلفية:** يعتبر الانسداد الوريدي العميق تجلطاً للدم فى وريد رئيسى ، عادة فى الأرجل أو فى منطقة الحوض . و عند انقطاع جزء من الجلطة تصبح انسداداً يمكن تحركه خلال القلب ليسد شرايين الرئة. يعتبر العامل الخامس لايدن عامل وراثى خطير لاضطراب زيادة التجلط المورث فى كثير من الشعوب. تحقق الدراسة الحالية فى ارتباط طفرة العامل الخامس لايدن بالانسداد الوريدي العميق بين حالات مصرية.

**الحالات و الطرق:** تتضمن الدراسة 44 حالة (16 ذكر و 28 انثى) بمدى عمرى من 20 الى ثمانين عاماً بالاضافة الى 211 شخص أصحاء كمجموعة ضابطة بعمر و جنس متوافق. تم استخدام افاضة ال DNA بتفاعل البلمرة المتسلسل الخاص بالصورة الجينية للكشف عن الطفرة الجينية للعامل الخامس لايدن (G1691A) .

**النتائج:** أظهرت الحالات الصورة الجينية المتطفرة A (طرز جينية AA و AG) أكثر بصورة ذات دلالة عن المجموعة الضابطة ( $P < 0.05$ , OR= 2.78 and CI 1.380–5.589, 95%).

**الاستنتاج:** تنتهى النتائج الى أن طفرة العامل الخامس لايدن تتكرر بصورة عالية و ترتبط ارتباطاً ايجابياً بظهور الانسداد الوريدي العميق بين الحالات المصرية.