ORIGINAL PAPER

Nagoya J. Med. Sci. **83**. 407–417, 2021
doi:10.18999/nagjms.83.3.407

The prevalence of Factor V Leiden (Arg506Gln) mutation in King Khalid University Hospital patients, 2017–2019

Maram Al-Otaiby[1], Rahaf Althnayan[2], Alanoud Binmethem[2], Reema Bader AlEnezy[2], Munira Abdulrahman Alhadlg[2], Arjuwana Alaqeel[2], Sara H AlQahtani[2], Noman Ghufran[1], Abdulaziz A. Alotaibi[3], Nada Alayed[2] and Imran Ali Khan[4]

[1]*Molecular Genetic Pathology Unit, Pathology Department, King Saud University, Riyadh, Saudi Arabia*
[2]*College of Medicine, King Saud University, Riyadh, Saudi Arabia*
[3]*Public Administration Department, College of Business, King Saud University, Riyadh, Saudi Arabia*
[4]*Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia*

ABSTRACT

Arg506Gln mutation is responsible for one of the procoagulant factors and most common inherited thrombophilia in the Factor V Leiden (FVL) family. The replacement of the missense mutation for Arg506Gln / R506Q is at 1691st position from Guanine to Adenine with the modification of the amino acid from arginine to glutamine. The aim of this study was to investigate the current prevalence of the G1691A mutation in the FVL gene in the capital city's King Khalid University Hospitals (KKUH). Since 2017–2019 we have recruited 482 patients in these cross-sectional studies to test the G1691A mutation in KKUH's FVL gene. DNA was extracted using 2mL of the EDTA blood and genotyping was performed with polymerase chain reaction and the data was analyzed using Sanger sequencing. In this study, 4.4% of the G1691A mutation was found to be positive (combined heterozygous-GA and homozygous-AA variants) and 95.6% of them with negative, i.e., homozygous normal-GG genotypes. Our study concludes that with the advances in genetic testing and their recent availability, early mutation detection could approve the genotype risks for many patients and this mutation is not as rare as previously believed in the Saudi region as our study has established with a 4.4 percent prevalence.

Keywords: Factor V Leiden (FVL), G1691A mutation, Arg506Gln, R506Q, KKUH

Abbreviations:
FVL: factor V Leiden
KKUH: King Khalid University Hospital
FVa: factor V, activated
FXa: factor X, activated
APC: activated protein C
VTE: venous thromboembolism
DVT: deep venous thrombosis
PE: pulmonary embolism
BMI: body mass index
PCOS: polycystic ovarian syndrome

Received: August 5, 2020; accepted: November 19, 2020
Corresponding Author: Imran Ali Khan, MSc, PhD
Clinical Laboratory Sciences Department, College of Applied Medical Sciences, King Saud University, Riyadh 11433, Kingdom of Saudi Arabia
Tel: +966-0-501112806, E-mail: imkhan@ksu.edu.sa
ORCID: 0000-0002-9746-5300

INTRODUCTION

Factor V is one of the procoagulant factors of the clotting cascade, activated factor V (FVa) together with activated factor X (FXa) convert prothrombin to its active form thrombin, and that ultimately leads to the clot formation. FVa is then degraded by activated protein C, which is a natural anticoagulant that cleaves FVa at arginine R-506, R306 and R679 in the heavy chain.[1-3] The replacement of glutamine (Q) for R506 results in a single point mutation known as Factor V Leiden (FVL) represented as R506Q. This mutation revokes the cleavage site for activated protein-C (APC) and gives rise to a poor anticoagulant response to it, therefore reducing the degradation rate of FVa, consequently allowing a prolonged prothrombinase complex activity.[4] The inheritance of the mutated gene may be either homozygous or heterozygous. In both types, there is an increased tendency of developing venous thromboembolism (VTE). According to a study done by Davidian et al,[1] Heterozygosity or heterozygous mutation carries a relative risk of incident VTE of approximately three-to-eight-fold. In contrast, homozygosity carries a relative risk of incident VTE of 80-fold. The clinical importance of this mutation lies in the growing evidence that patients with positive FVL have a markedly increased risk of VTE most commonly: deep venous thrombosis (DVT) and pulmonary embolism (PE). Although normal pregnancy is associated with a five-to-tenfold increased risk of developing VTE, this risk is remarkably higher in women who are positive for the FVL mutation, in addition to its association with recurrent fetal loss.[5] There are two ways for the detection of FVL mutation. The first is an APC resistance test, and the second method being direct DNA based genotyping. Patients who undergo the APC resistance test and their results turn out to be positive, further confirmation is needed through the DNA methods. This step is imperative as the APC resistance testing lacks the ability to distinguish between homozygous and heterozygous individuals.[6,7] This distinction is of great importance clinically since homozygous individuals have about a 10-fold increase in the risk of thrombotic events than their heterozygous counterparts.[8]

The utility of research on the FVL mutation has not been clearly understood for a long time. Opinions and practices concerning FVL testing varied considerably among doctors. To overcome this dilemma, the American College of Medical Genetics has developed some guidelines to direct doctors on when to assume an inherited thrombophilic condition and order the correct test for it. It is recommended that the FVL test be ordered in any of the following circumstances: 1-First VTE before 50 years of age, 2-First VTE above 50 years of age in the absence of malignancy, 3-venous thrombosis in unusual sites (such as hepatic, mesenteric and cerebral veins), 4-recurrent VTE, 5-first VTE and a strong family history of VTE, 5-VTE during pregnancy, postpartum or women. On the other hand, general population random screening for FVL, prenatal testing, and routine screening of newborns are not all recommended.[8] Although there are numerous causes of inherited thrombophilia, resistance to activated protein C, and more specifically the FVL mutation, has proved to be the most prevalent inherited risk factor for venous thromboembolism. The prevalence of the FVL mutation varies widely around the world, being the highest among Caucasians of European descent.[9] While, Jordanians have the highest frequency among Arab populations ranging from 10.5%–27.5% of the general population.[10-12] On the contrary, there were lower numbers in the gulf region with a prevalence of 3%–14% among Bahrainis, and 0%–2% among Saudi Arabs.[13]

The prevalence of FVL in Arab cultures, and more specifically the population of Saudi Arabia, remains ambiguous and obsolete in the literature. For this purpose, through our study, we aim to

estimate the current prevalence of FVL mutation in King Khalid University Hospital (KKUH), which will give us an insight into the general distribution of the mutation in the country and its relationship with different demographics, as well as the magnitude of its association with numerous clinical features, mainly venous thromboembolism and unexplained misc. While these thrombotic events carry high morbidity and mortality rates, they are also fairly easily avoided if doctors have a clear idea of when to suspect the mutation, order the test, and provide the necessary prophylaxis to potentially improve patient outcomes.

MATERIALS AND METHODS

Ethical approval

The ethical grant for this study was obtained from the King Saud University (E-19-4445) Board of Institutional Review. The patients involved signed up to this study with the informed consent form. None of the patients have earned any incentive to participate in this study and none of the patients have any conflict of interest for this study.

Patient recruitment

It is an observational longitudinal, systematic cross-sectional analysis carried out from October 2019 until April 2020 at KKUH Riyadh, Saudi Arabia. The study included all patients screened for 2017 to 2019 FVL (Arg506Gln) mutation. A minimum sample size based on 9 was determined using a single proportion standard equation (95% confidence interval). The research included an appropriate number of 482 patients, after applying the criteria for inclusion and exclusion. All of those patients' medical file numbers were demanded from the department of molecular genetics. Then genetic reports of the patients were collected from electronic records to collect mutation test results, as well as demographic and clinical data relevant to the study. Full data were recorded in a datasheet for transfer. Results for clinical and demographic details were obtained. Patients were tested for FVL mutation in KKUH from 2017–2019 and the exclusion criteria were patients positive for FII mutation, and positive or negative for FVL mutation. Another exclusion criteria for this study were lack of medical records.

Sample collection and molecular analysis

2mL of the peripheral blood was transferred to EDTA vacutainer from each and every patient, and DNA was extracted and was used for molecular analysis. NanoDrop was used to calculate quantification of DNA, and to research the Arg506Gln mutation in the FVL gene polymerase chain reaction was performed. Details of the primers opted, analysis of genotyping and Sanger sequencing was shown in the previous studies.[4,9-11]

Data Analysis

Data were analyzed using statistical tools for the version SPSS 24.0. The quantitative and categorical variables were defined using descriptive statistics (mean, standard deviation, concentrations, and percentages). The Chi-square test by Pearson was used to assess the significance between the findings of the FVL mutation test and the different categorical variables. The univariate likelihood ratio was calculated using coefficients for logistic regression. The statistical significance and accuracy of the results were stated using a p-value of < 0.05 and 95% CI. Multivariate logistic regression models for gender, nationality, Body Mass Index (BMI) and DVT were used to determine the adjusted odds ratio.

RESULTS

482 patients tested for FVL mutation were subjected to statistical analysis, which determined different demographic and clinical distributions. The first demographic data to be analyzed was age, 37 years on average and 13.66 standard deviation. The minimum age was 6 months while the mean age was 82. In addition, the check for females was ordered 76.1% more frequently than for males' 23.9% and for Saudis 83.4% more frequently than for non-Saudis 16.6% (Tables 1). The clinical data were subsequently analyzed starting with comorbidities, 13.1% of those studied had diabetes. Though 11.8% had hypothyroidism and 10.0% had hypertension. Though dyslipidemia, anemia and polycystic ovarian syndrome (PCOS) cases were significantly lower at 5.4%, 2.7% and 4.6% respectively. A high percentage of patients tested had 26.6% of DVT, while 13.7% had strokes and 9.3% had PE. Most notable, however, was that 44.4% of the female patients screened for FVL mutation suffered from miscarriages. The distribution of the BMI among the sample was as follows: 3.3% of the study sample was underweight, 26.8% was of normal weight, 29.4% was overweight, 18.7% were of Class 1 obesity, 12.6% were of Class 2 obesity and 9.2% were moderate obesity (Table 1).

Table 1 Socio demographics and clinical characteristics among patients tested for factor V Leiden mutation (n=482)

Variables	Frequency % (n)
Gender	
Female	76.10 (367)
Male	23.90 (115)
Nationality	
Saudi	83.40 (402)
Non-Saudi	16.6 (80)
Comorbidities	
Diabetes Mellitus	13.1 (63)
Hypertension	10.0 (48)
Dyslipidemia	5.4 (26)
Anemia	2.7 (13)
Polycystic Ovary Syndrome (PCOS) (n=367)	4.6 (17)
Hypothyroidism	11.8 (57)
Clinical Manifestation	
Deep Vein Thrombosis (DVT)	26.6 (128)
Pulmonary Embolism (PE)	9.3 (45)
Stroke	13.7 (66)
Miscarriage (n= 367)	44.4 (163)
Body Mass index (BMI) (N=456)	
Underweight (less than 18.5)	3.30% (15)

Normal (18.6–24.9)	26.8(123)
Overweight (25–29.9)	29.4(135)
Class 1 Obesity (30–34.9)	18.7(86)
Class 2 Obesity (35–39.9)	12.6(58)
Morbid Obesity (>40)	9.2(42)
Variable	Mean (sd)
Age	37.66 (13.66)
	Maximum 82 Years
	Minimum 6 Months

Table 2 showed the number of positive cases of FVL (a total of 21 positive cases) comprising 4.4% of the sample. Twenty of which had a heterozygous mutation, and only one had a homozygous mutation. The mean age of positive FVL mutation, with a standard deviation of 13.69, was 37.48. The minimum age was eight years while the maximum was sixty-one. Of those who tested positive 57.1% were female and 42.9% were male, with a p-value of 0.037 for correlation. 66.7% of mutation patients were non-Saudi, with a p-value correlation of < 0.001. The most common comorbidities were diabetes mellitus (p-value>0.05), and dyslipidemia (p-value>0.05), each affecting 14.3%. Less common comorbidities included 9.5% hypertension, 8.3% PCOS, 4.8% hypothyroidism and 0% anemia, all with > 0.05 p-value association. With respect to clinical manifestations, DVTs suffered 47.6%, with a (p=0.025) correlation. Though 14.3% had strokes (p-value > 0.05). But most interestingly, PE (p-value>0.05) or miscarriage was encountered by 0%. With the latter having a p-value of 0.002 for correlation. The distribution of BMI among the positive cases of FVL mutation was as follows: 5.9% underweight, 11.8% normal weight; 23.5% overweight, 17.6% Class 1 obesity, 23.5% Class 2 obesity, 17.6% Morbidly obesity. For BMI the p-value for the correlation is 0.009.

Table 2 Factor V Leiden mutation

Variable	Frequency % (n)	
Factor V Leiden mutation (n=482)		
Positive	4.4 (21)	
Negative	95.6 (461)	
Distribution of Socio Demographics and Clinical Characteristics among Positive FVL Mutations (n=21)		
Variable	Frequency %	(p-value)
Zygosity		
Heterozygous	95.2 (20)	
Homozygous	4.8 (1)	
Gender		0.037
Female	57.1(12)	
Male	42.9(9)	
Nationality		<0.001
Saudi	33.3(7)	
Non-Saudi	66.7(14)	

Comorbidities		
Diabetes Mellitus	14.3 (3)	0.866
Hypertension	9.5 (2)	0.946
Dyslipidemia	14.3 (3)	0.065
Anemia	0 (0)	0.435
PCOS (N=12)	8.3 (1)	0.535
Hypothyroidism	4.8(1)	0.305
Clinical Manifestation		
DVT	47.6(10)	0.025
PE	0(0)	0.133
Stroke	14.3(3)	0.936
Miscarriage (n=12)		
	0(0)	0.002
Body Mass index (N=17)		0.009
Underweight (less than 18)	5.9 (1)	
Normal (18.5–24.9)	11.8 (2)	
Overweight (25–29.9)	23.5 (4)	
Class 1 Obesity (30–34.9)	17.6 (3)	
Class 2 Obesity (35–39.9)	23.5 (4)	
Morbid Obesity (>40)	17.6 (3)	

Variable	Mean (sd)
Age	37.48 (13.69)
	Minimum 8 years
	Maximum 61 years

Table 3 examines the association between FVL and the various socio-demographic and clinical features. Firstly, the univariate analysis concluded that there is a statistically significant association between FVL and gender, nationality, BMI and the occurrence of DVT. There were no significant associations with the other populations and clinical characteristics (age, diabetes mellitus, hypertension, dyslipidemia, hypothyroidism, anemia, ovarian polycystic syndrome, pulmonary embolism, stroke, and miscarriage). Sex was listed also as lacking of significant factors. With the odds of having FVL mutation significantly lower in females than males (odds ratio = 0.398, confidence interval of 95% CI = 0.163–0.971; p = 0.043). As well as race playing a role showing itself to be a protective factor with an odds ratio of 0.084 (95% CI = 0.033–0.215; p < 0.0001). There was also a high association between FVL mutation and BMI (odds ratio = 1.056, 95% CI = 1.004–1.110; p= 0.034), and the association between FVL mutation and DVT incidence was extremely high with an odds ratio of 2.643 (95% CI = 1.094–6.381; p = 0.031) as expected. On multivariate analysis, only Saudi nationality (odds ratio = 0.075, 95% CI = 0.025–0.224; p < 0.001) and BMI (OR = 1.065, 95% CI = 1.007–1.126; p = 0.026) discerned a substantial association. However, there was a lower correlation with gender (odds ratio = 2.178, 95% CI = 0.728–6.519; p = 0.164) and the frequency of DVT (OR = 2.713, 95% CI = 0.917–8.029; p = 0.071) (Table 3).

Table 3 Association of FVL mutation with socio demographic and clinical characteristics

Variable	Odds Ratio	95% C. I		P-value
		Lower	Upper	
Univariate				
Female				
Male (reference)	0.398	0.163	0.971	0.043
Saudi	0.084	0.033	0.215	<0.0001
Non-Saudi (reference)				
Age	0.999	0.967	1.033	0.949
BMI	1.056	1.004	1.110	0.034
DM	0.898	0.257	3.140	0.866
HTN	0.950	0.214	4.207	0.946
Dyslipidemia	3.174	0.872	11.555	0.800
Hypothyroidism	0.362	0.048	2.747	0.326
Anemia	0.000	0.000	0.000	0.999
PCOS (n=367)	2.037	0.403	10.298	0.389
DVT	2.643	1.094	6.381	0.031
PE	0.000	0.000	0.000	0.998
Miscarriages (n=367)	0.000	0.000	0.000	0.995
Stroke	1.053	0.301	3.678	0.936
Multivariate				
Gender	2.178	0.728	6.519	0.164
Nationality	0.075	0.025	0.224	<0.001
BMI	1.065	1.007	1.126	0.026
DVT	2.713	0.917	8.029	0.071

Concluding with Table 4, it displays the distribution of ordering clinics across the entire sample when opposed to those with FVL positive. The vast majority of tests were conducted by the Internal Medicine clinics at 56.8% while analyzing the total sample, followed by 26.1% from the Clinics of Obstetrics and Gynecology and 5.2% from the Clinics of Surgery. Pediatrics had the fewest orders with a 3.3% ratio. Nevertheless, the distribution changes as compared with results drawn solely from the positive patients. Showing that the top three ordering clinics are as follows: Internal Medicine (66.7%), followed by surgery (14.3%), and finally Clinics in Obstetrics and Gynecology (9.5%). Most significantly, the dramatic percentage decrease from the Obstetrics and Gynecology clinics (from 26.1%–9.5%) is an estimated 1⁄3 decline, and the percentage increase within the surgical clinics (5.2% to 14.3%) is nearly 3-fold.

Table 4 Distribution of ordering clinics and clinical presentations among the sample and positive FVL

Distribution of Ordering Clinics		
Ordering Clinics	Frequency % (n)	
	Sample (n=482)	Positive FVL (n=21)
Internal Medicine	56.8 (274)	66.7 (14)

Obstetrics & Gynecology	26.1 (126)	9.5 (2)
Surgery	5.2 (25)	14.3 (3)
Pediatrics	3.3(16)	4.8 (1)
Other	8.5 (41)	4.8 (1)
Distribution of Clinical Presentations		
Clinical Presentations		
DVT	19.9 (96)	38.1 (8)
PE	6.2 (30)	9.5 (2)
Stroke	17.4 (84)	4.8 (1)
Miscarriage	16.0 (77)	0 (0)
Infertility	1.9 (9)	0 (0)
Pregnancy Follow-up	8.9 (43)	4.8 (1)
Retinopathy	3.3 (16)	0 (0)
Miscellaneous	26.3 (127)	28.6 (6)

Ultimately, Table 4 shows the main Clinical Test Presentations in contrast to those positive for FVL. The level of main presentations from the complete sample was as follows: the vast majority of patients with miscellaneous symptoms were 24.1% nearly one fourth, followed by 20.1% DVT, 17.4% stroke, 16% miscarriages, 8.9% pregnancy follow - ups, 6.2% PE, 3.3% retinopathy, and finally 1.9%, infertility follow - ups In comparison, the distribution of clinical presentations among positive FVL mutation patients shows a wide difference in results with the highest frequency of presentations being 38.1% of DVT, followed in 26.6% of PE, 9.5% of PE, 4.8% of stroke, and 4.8% of pregnancy follow - ups. But most notably, 0% had miscarriages, infertility and retinopathy.

DISCUSSION

The significance of this study was to determine the frequency of FVL mutation among the target population. It was found that the frequency of FVL mutation is 4.4% of all cases. Only a third of these patients were of Saudi nationality while the majority were non-Saudi. The most recent study on the Saudi population was a 2012 study by Settin et al[12] which found the frequency of FVL mutation in 2.2% of their study sample. A 37-year old female patient confirmed the homozygous variant that had visited the out-patient clinic in KKUH. This difference is most likely due to the fact that their sample included 352 healthy participants all of Saudi nationality, which compromised of blood donors and university employees. However, since this study encompasses the Saudi population and not just those of Saudi nationality exclusively, as well as the participants being hospital patients, a larger frequency of the mutation was observed. There is a large variation in the frequency for FVL mutation in the Middle Eastern region alone. For instance, a high prevalence was observed in Jordan 23.5%,[13] Lebanon 12.1%[14] and Iraq 7.4%.[15] Meanwhile, lower frequencies were noted in Kuwait 4.5%[13] and Algeria 2%.[16] In comparison, the prevalence concluded from this study is compatible with that of the surrounding region, albeit on the lower side.

Internationally FVL mutation has always been associated with European and Caucasian populations. For instance, a high prevalence can be appreciated in Sweden 10–15%,[5] Greece 16.8%[17] and

Italy 9.5%.[18] While on the other hand, frequencies were extremely low in most Asian populations, with India at 1.3%[8] and 0% reported in both Korea[19] and China.[20] The previous figures indicate that the prevalence of FVL in the Saudi population, as well as in the Middle Eastern region, is higher than expected, and conforms more accurately to the Caucasian/European population than the Asian populous. In the study to evaluate demographic and medical associations, two groups were established to draw conclusions from by comparison of their statistical results. The first being the entire sample of patients tested for the FVL mutation, and the smaller group comprised solely of those who tested positive. Beginning with the demographic datum. The mean age of detection for the complete sample is 37 years, with a range between (6 months- 82 years). Similar results were seen in the smaller group of those who tested positive with a mean of 37.48 and a narrower range of (8–61). The peak age of detection was 34.5 years in the most recent international study conducted in 2019.[21] Globally, testing for FVL mutation is more commonly ordered for female patients rather than male, with a study conducted in Australia reporting more than 70% of requests were for female patients. This pattern is also seen in our study with 76.1% of orders being requested for female patients. However, this gender bias is not supported when compared to the proportionate gender distribution. As stated by our findings, male gender prevalence was more than double female prevalence. With the study conducted in Australia yielding similar results, unfortunately, this variable was not taken into consideration by studies in the region.[21]

Various comorbidities were identified to be examined to examine whether a link could be made between them and the FVL mutation; these included co-morbidities (diabetes, hypertension, hypothyroidism, polycystic ovary syndrome and anemia). The results were quite similar while their concentrations were compared between the two groups, with no significant trends found. Nevertheless, one significant finding was found that the prevalence of dyslipidemia between the two groups increased from 4.6% in the entire sample to 14.3% of those surveyed. Considering that the FVL mutation was only under investigation for less than two decades, no research could be correlated with these findings. The largest percentage of those who encountered DVTs were found to be 26.6% as predicted when researching a thrombophilic mutation, after analyzing the clinical manifestations within the entire sample, and 9.3% experienced PE's. As a result, 35.9% of males and females are diagnosed with thromboembolisms. A percentage that resembles but slightly lower than that of an American study that reported a collective 39.4%.[21] In our positive patient group, 47.6% reported DVTs, most notably the absence of PE in this category with a 0% outcome.

The last of all clinical manifestations to be examined was pregnancy and fetal morbidity at 44.4% of all female requests, with a striking contrast to the 16.4% found in the American study.[21] A perhaps even more stunning finding is shown compared to the positive group of which no women had an amazing 0% pregnancy / fetal morbidity. In the medical community there is apparent uncertainty as to what appearance merits an ordered FVL test, and this is also seen in our own findings. Stressing the importance of following recent guidelines when ordering a test. Of the complete sample, the highest percentage with miscellaneous symptoms was 24.1%, followed by 20.1% with DVT, 17.4% with stroke, 16% with miscarriages, 8.9% follow - ups with pregnancy, and last but not least 1.9% with infertility. Compared to the positive group's primary presentation, patients with DVTs appear as a clear majority at 38.1%, follow-ups of pregnancy at 4.8%. Interestingly 0% had infertility or retinopathy. But most important of all perhaps none of the positive females had miscarriage as their primary presentation. Distribution of ordering clinics revealed the suspected by the Obstetrics and Gynecology clinics over ordering. Expressed when we compare orders from these clinics between the whole sample and only the positive ones. Of the entire sample, 26.1% of the test orders originated in the positive group from the

obstetrics and gynecology clinics with a fall to 9.5%.

This study has certain limitations and weaknesses: there are still some inevitable complications, despite the fact that this research has met its objectives. First, a multi-center research could not have been conducted due to a limited time frame, since a nationwide sample may have produced different results and indicate a variation in the prevalence of the FVL mutation between different regions of Saudi Arabia. Second, incomplete secondary data might have been obtained for each patient as a result of fragmented and inadequate documentation. Finally, specific nationalities of non-Saudi patients may have indicated the spread of the mutation in Saudi Arabia among the various ethnicities. The lack of data on treatment was another limitation of this study.

CONCLUSION

With the advances in genetic testing and their recent availability, early mutation detection could confirm the genotype risks for many patients and this mutation is not as rare as previously believed in the Saudi region as our study has demonstrated with a 4.4% prevalence. During the age of genetic testing, targeted patient selection surfaces of great importance cannot be established efficiently or effectively without establishing a clear structure for test ordering. Our data highlights the non-compliance with a specified criterion when ordering the FVL test. The strong difference in ordering for female patients sets this out. This seems to derive from the idea that this mutation can trigger unexplained miscarriages, but our study warrants questioning whether miscarriages are significantly linked to this mutation. We also recommend that the recently developed recommendations be strictly adhered to by physicians and routinely applied.

CONFLICT OF INTEREST

There is no conflict of interest for this study.

REFERENCES

1 Benjamin T, Davidian MH, DeSimone EM 2nd. Review of factor V Leiden thrombophilia. *US Pharm*. 2018;43(5), (Specialty&Oncology suppl):12–15.

2 Shaya SA, Westrick RJ, Gross PL. Thrombus stability explains the factor V Leiden paradox: a mouse model. *Blood Adv*. 2019;3(21):3375–3378. doi:10.1182/bloodadvances.2019031112

3 El-Ghonemy MS, El Sharawy S, Fahmi MW, El-Ashwah S, Denewer M, El-Baiomy MA. Thrombophilic Risk of Factor V Leiden, Prothrombin G20210A, MTHFR, and Calreticulin Mutations in Essential Thrombocythemia Egyptian Patients. *Adv Hematol*. 2020;2020:7695129. doi:10.1155/2020/7695129

4 Khan IA, Shaik NA, Kamineni V, Jahan P, Hasan Q, Rao P. Evaluation of Gestational Diabetes Mellitus Risk in South Indian Women Based on MTHFR (C677T) and FVL (G1691A) Mutations. *Front Pediatr*. 2015;3:34. doi:10.3389/fped.2015.00034

5 Kujovich JL. Factor V Leiden thrombophilia. *Genet Med*. 2011;13(1):1–16. doi:10.1097/GIM.0b013e3181faa0f2

6 Campello E, Spiezia L, Radu CM, Simioni P. Microparticles as biomarkers of venous thromboembolic events. *Biomark Med*. 2016;10(7):743–755. doi:10.2217/bmm-2015-0063

7 Grody WW, Griffin JH, Taylor AK, Korf BR, Heit JA; ACMG Factor V. Leiden Working Group. American College of Medical Genetics consensus statement on factor V Leiden mutation testing. *Genet Med*. 2001;3(2):139–148. doi:10.1097/00125817-200103000-00009

8 Herrmann FH, Koesling M, Schrŏder W, et al. Prevalence of factor V Leiden mutation in various populations. *Genet Epidemiol*. 1997;14(4):403–411. doi:10.1002/(SICI)1098-2272(1997)14:4<403::AID-GEPI5>3.0.CO;2-3

9 Khan IA, Movva S, Shaik NA, et al. Investigation of Calpain 10 (rs2975760) gene polymorphism in Asian Indians with Gestational Diabetes Mellitus. *Meta Gene*. 2014;2:299–306. doi:10.1016/j.mgene.2014.03.001

10 Kamineni V, Khan IA, Vattam KK, et al. Influence of thrombophilic genes; MTHFR (C677T), FVL (G1691A) and ACE (I28005D) in pregnant women with pre-eclampsia. *Obstet Gynecol Int J*. 2015;2(1):14–20. doi:10.15406/ogij.2015.02.00023
11 Khan IA, Jahan P, Hasan Q, Rao P. Genetic confirmation of T2DM meta-analysis variants studied in gestational diabetes mellitus in an Indian population. *Diabetes Metab Syndr*. 2019;13(1):688–694. doi:10.1016/j.dsx.2018.11.035
12 Settin AA, Alghasham A, Ali A, Dowaidar M, Ismail H. Frequency of thrombophilic genetic polymorphisms among Saudi subjects compared with other populations. *Hematology*. 2012;17(3):176–182. doi:10.1179/102453312X13376952196575
13 Dashti AA, Jadaon MM, Lewis HL. Factor V Leiden mutation in Arabs in Kuwait by real-time PCR: different values for different Arabs. *J Hum Genet*. 2010;55(4):232–235. doi:10.1038/jhg.2010.11
14 Zahed LF, Rayes RF, Mahfouz RA, Taher AT, Maarouf HH, Nassar AH. Prevalence of factor V Leiden, prothrombin and methylene tetrahydrofolate reductase mutations in women with adverse pregnancy outcomes in Lebanon. *Am J Obstet Gynecol*. 2006;195(4):1114–1118. doi:10.1016/j.ajog.2006.06.082
15 Dashti AA, Jadaon MM. Race differences in the prevalence of the factor V Leiden mutation in Kuwaiti nationals. *Mol Biol Rep*. 2011;38(6):3623–3628. doi:10.1007/s11033-010-0474-7
16 Bourouba R, Houcher B, Djabi F, Egin Y, Akar N. The prevalence of methylenetetrahydrofolate reductase 677 C-T, factor V 1691 G-A, and prothrombin 20210 G-A mutations in healthy populations in Setif, Algeria. *Clin Appl Thromb Hemost*. 2009;15(5):529–534. doi:10.1177/1076029608319944
17 Hatzaki A, Anagnostopoulou E, Metaxa-Mariatou V, et al. The impact of heterozygosity for the factor V Leiden and factor II G20210A mutations on the risk of thrombosis in Greek patients. *Int Angiol*. 2003;22(1):79–82.
18 Sottilotta G, Mammì C, Furlò G, Oriana V, Latella C, Trapani Lombardo V. High incidence of factor V Leiden and prothrombin G20210A in healthy southern Italians. *Clin Appl Thromb Hemost*. 2009;15(3):356–359. doi:10.1177/1076029607310218
19 Kim TW, Kim WK, Lee JH, et al. Low prevalence of activated protein C resistance and coagulation factor V Arg506 to Gln mutation among Korean patients with deep vein thrombosis. *J Korean Med Sci*. 1998;13(6):587–590. doi:10.3346/jkms.1998.13.6.587
20 Ho CH, Chau WK, Hsu HC, Gau JP, Chih CM. Prevalence of factor V Leiden in the Chinese population. *Zhonghua Yi Xue Za Zhi (Taipei)*. 1999;62(12):875–878.
21 Favaloro EJ. Genetic testing for thrombophilia-related genes: observations of testing patterns for factor V Leiden (G1691A) and prothrombin gene "Mutation" (G20210A). *Semin Thromb Hemost*. 2019;45(7):730–742. doi:10.1055/s-0039-1694772