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Original Article

Prevalence of Factor V Leiden Mutation in Healthy Females of Sindh, Pakistan and Comparison of Three Detection Methods in Resource-Limited Settings

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ABSTRACT

Factor V Leiden (FVL) mutation is related to venous thromboembolism and pregnancy complications. It is highly prevalent among Caucasians; however, few studies have been conducted on the Asian population. **Objective:** To find the prevalence, genotype, and allelic frequency of FVL mutation in females of Sindh, Pakistan, and to develop a valid and economical method for the detection of FVL mutation in resource-limited settings. **Methods:** Hundred (n=100), unrelated healthy females of Sindh, Pakistan, were recruited. FVL was detected using three methods, i.e., tetra primer amplification refractory mutation system (ARMS)-polymerase chain reaction (PCR), PCR-restriction fragment length polymorphism (PCR-RFLP), and PCR-sequencing. Prevalence, genotype, and allele frequencies were calculated. We compared the three mutation detection methods to find a suitable protocol for FVL detection in developing countries. **Results:** The prevalence of FVL mutation was 3% in healthy females of Sindh, Pakistan. No homozygous FVL mutation was found. The results from tetra primer ARMS-PCR, PCR-RFLP, and PCR-sequencing were 100% concordant. Tetra primer ARMS-PCR was a valid, reliable, faster, economical genotyping and screening method. **Conclusions:** In conclusion, FVL mutation was found in our population, and further studies should be conducted to find its role in thromboembolic and obstetrical complications. In addition, we have suggested tetra primer ARMS-PCR as an appropriate method for FVL detection in resource-limited settings.

INTRODUCTION

Inherited or acquired thrombophilia increases the risk of thromboembolism. The factor V Leiden (FVL) mutation, the factor II gene G20210A mutation, methylenetetrahydrofolate reductase (MTHFR) gene C677T mutation, and insufficient antithrombin, protein C and protein S are common inherited causes [1, 2]. Acquired thrombophilias are frequent in patients undergoing surgery, injury, longstanding immobilization, older age, cancers, and pregnant women [3]. Coagulation factor V (FV) protein of the coagulation system circulates in an inactive form until activation of the coagulation cascade by blood vessel injury. It has procoagulant and anticoagulant

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characteristics. Its deficiency can lead to mild bleeding or even life-threatening complications. The FV gene mutation may alter its function in the coagulation cascade [4]. The FV gene maps to 1q23-24 comprising 25 exons and 24 introns [5]. The FVL mutation is a missense single nucleotide polymorphism (SNP) in exon 10, causing substitution of a nucleotide at 1691 (adenine for guanine), changing amino acid from arginine to glutamine [6]. FVL mutation has shown an association with venous thromboembolism and pregnancy complications such as preeclampsia, stillbirth, recurrent miscarriage, and placenta abruption. Its association with obstetric complications increases its significance in females [7, 8]. Various protocols based on hybridization, enzymatic procedures, post-amplification methods, and sequencing have been used to detect FVL mutation [9]. However, choosing a suitable method for genotyping SNPs in developing countries with limited resources and financial constraints is essential.

METHODS

A cross-sectional study was conducted at Molecular and Genetics Laboratory, Medical Research Centre (MRC), Liaquat University of Medical & Health Sciences (LUMHS) Jamshoro after taking Ethical approval between the years 2014 and 2015. For this study, 100 unrelated adult healthy females were recruited from general population of Sindh by using non-probability convenience sampling method. The sample size was calculated by taking 5% prevalence of FVL mutation from previous studies [10], using OpenEpi software (https://www.openepi.com/SampleSize). The minimum sample size obtained was n = 73 at 95% confidence level, however to increase study power it was increased to n = 100. The inclusion criteria were female participants, born and residing in the Sindh province of Pakistan, aged between 18 and 45 years, without a known personal and family history of thromboembolic and hematological disorders. Participants with a history of chronic medical diseases such as hypertension, chronic renal diseases, diabetes mellitus, and thrombotic obstetric complications and disorders were excluded. Five ml of venous blood was withdrawn from participants after obtaining written informed consent by the routine method under aseptic measures. Blood was transferred into a falcon tube containing 400 µl of anticoagulant ethylenediaminetetraacetic acid 0.5M (EDTA). Samples were stored at -80°C till analyzed. DNA extraction was performed by inorganic method. Optical density (OD) was measured by DU-800 UV-visible spectrophotometer for quality assessment and quantification. DNA products were separated on 0.8% agarose gel (Figure 1A).

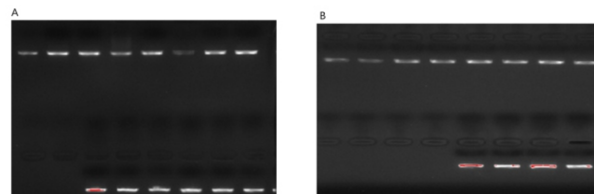

Figure 1: Agarose gel electrophoresis (A) For genomic DNA (B) For PCR products

FVL mutation detection by PCR-RFLP involved MnlI as a restriction enzyme. According to the previously published study, forward and reverse primers and amplification conditions were as follow: PCR reaction mixture of 25µl was prepared according to the following protocol: $MgCl_2$ buffer (2.5mM) 2.5µl, dNTPs (1.25mM) 2.5µl, Taq polymerase 0.6µl, forward primer (8µM) 0.5µl, reverse primer (8µM) 0.5µl, DNA 4µl, and distilled deionized water 14.4µl [11]. After amplification and separation of PCR products on a 1.2% agarose gel (Figure 1B), PCR products were digested by restriction enzyme MnlI (Thermo scientific). Following constituents prepared a reaction mixture of 31 µl: PCR reaction mixture 10 µl, nuclease-free water 18 µl, 10X buffer G 2 µl, and MnlI 1 µl. PCR tubes were incubated at 37°C for 16 hours in 2720 thermocycler. MnlI digested PCR products separated upon 2% agarose gel (Figure 2). The PCR procedure produces amplification of a 287 bp fragment. Restriction with MnlI has bands of different sizes according to genotype as G/G yielded three bands of 37 bp, 93 bp, and 157 bp, A/A 2 bands of 130 bp and 157 bp and G/A 4 bands of 37 bp, 93 bp, 130 bp, and 157 bp sizes.

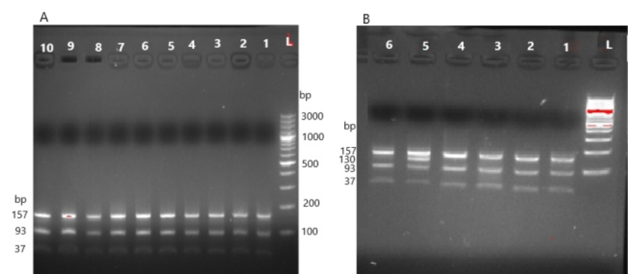

Figure 2: A and B. Agarose gel electrophoresis for separating MnlI digested PCR products

L= Ladder, Heterozygous (No.5) GA 4 bands: 37 bp, 93 bp, 130 bp, and 157 bp Homozygous GG 3 bands: 37 bp, 93 bp, and 157 bp

Detection of factor V Leiden by tetra primer ARMS-PCR was carried out in a single reaction and requires four primers, i.e., two inner and two outers. A previous study describes the design and validation of the tetra primer ARMS-PCR assay for FVL mutation [12]. Amplification of the 385 bp fragment yielded segments of the following sizes according to the genotype (Figure 3): G/G produced two bands of 385 bp and 254 bp, A/A had two bands of 385 bp and 184 bp, and G/A produced three bands of 385 bp, 254 bp, and 184 bp.

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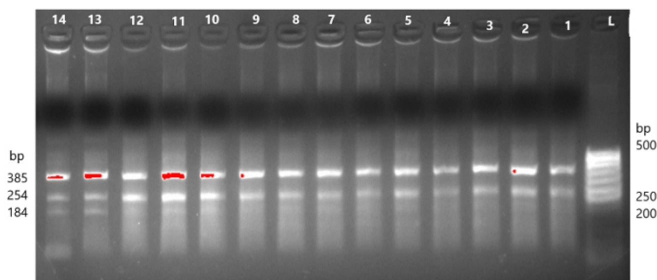

Figure 3: Agarose gel electrophoresis for the separation of PCR products via tetra primer ARMS-PCR

Sequencing was performed by the Sanger method using ABI 3130 Genetic Analyzer (Figure 4). Sequences from participants were compared with wild-type sequences by using the BLAST web tool provided by the National Center for Biotechnology Information USA.

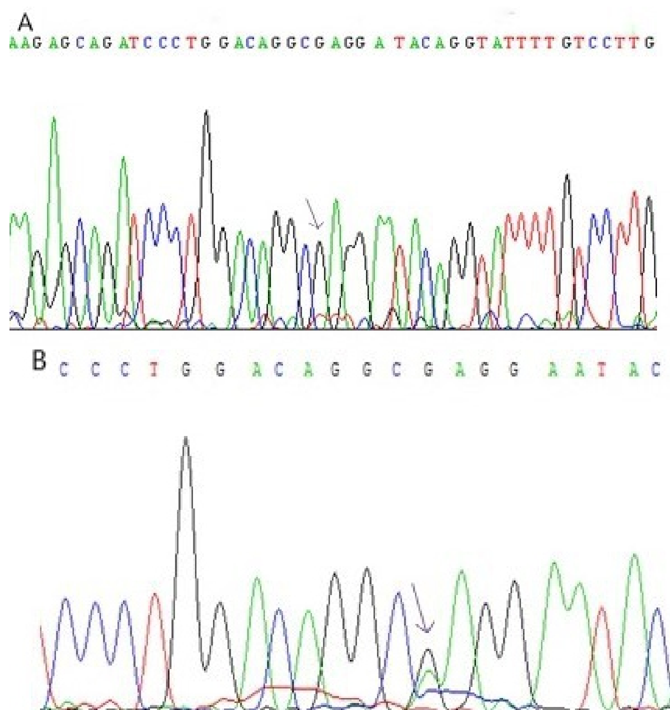

Figure 4: PCR-sequencing chromatograms (A) Homozygous GG (B) Heterozygous GA

The data were analyzed using Microsoft Excel. Mean age, prevalence, genotype frequency, and allele frequency were calculated. The Ethical Committee approved the current study of the Liaquat University of Medical and Health Sciences, Jamshoro. Informed consent was obtained from each participant.

RESULTS

The mean age of participants was 21 years. FVL mutation was detected in 3 out of 100 healthy females, indicating the prevalence of 3% of our female population. Among 100 participants, three were found heterozygous for FVL mutation. Our study showed no homozygous AA genotype detection for FVL mutation (Table 1, Figure 5).

Table 1: Genotype and allele frequency of factor V Leiden among participants

Genotype/Alleles	N (%)
GG	97 (97)
GA	03(3)
AA	00(0)
G	197(98.5)
A	03(1.5)

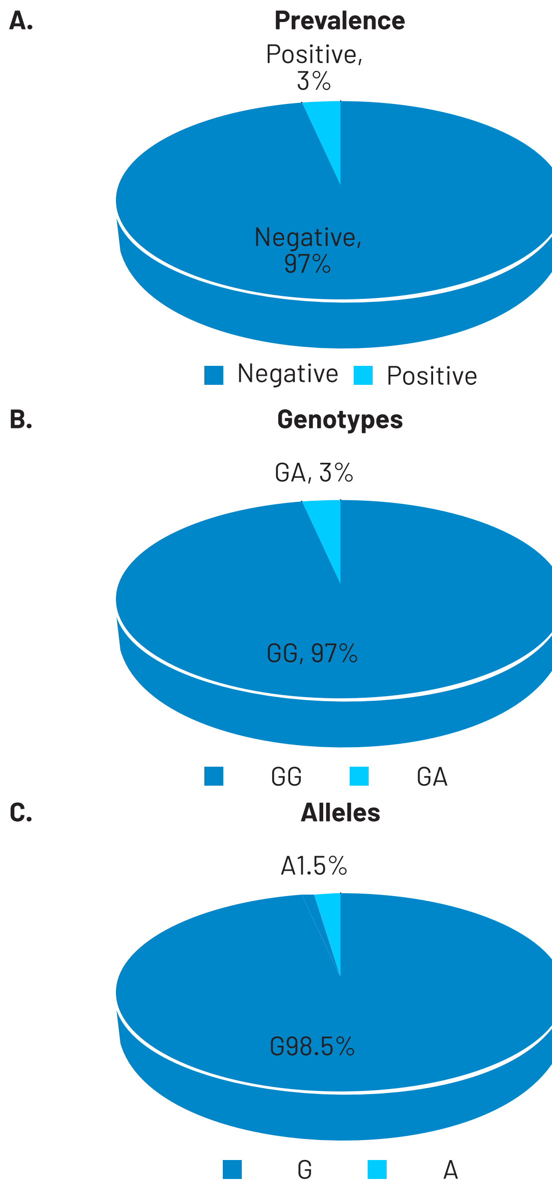

Figure 5: (A) Prevalence, (B) Genotype frequency, and (C) Allele frequency of factor V Leiden (FVL) mutation

Genotyping of FVL mutation by PCR-RFLP and tetra primer ARMS-PCR was concordant with PCR-sequencing in all samples, illustrating 100% concordance of all three methods for genotyping. PCR-RFLP and tetra primer ARMS-PCR methods prove to be 100% sensitive and specific for FVL mutation when compared with the gold standard confirmatory method of PCR

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sequencing.

Table 2 compares the three methods for cost, turnaround time, and analysis of a total number of samples simultaneously for FVL detection. Tetra primer ARMS-PCR was found as a cost-effective and fast method for detecting FVL mutation compared to PCR-RFLP and PCR-sequencing.

Table 2: Comparison of three methods for detection of factor V Leiden

Method	Cost	Turnaround time	Number of samples analyzed at a time
PCR-RFLP	7 USD	16 h 43 min	96
Tetra primer ARMS-PCR	4.5 USD	1 h 53 min	96
PCR sequencing	18 USD	2 days	04

DISCUSSION

FVL has been related to thrombotic obstetric complications, and its screening has been given importance in females of reproductive age and on oral contraceptive pills [13, 14]. Our study reports a 3% prevalence of FVL mutation in healthy adult females of Sindh, Pakistan, which is comparatively higher than the prevalence detected in adult healthy males from Punjab (1%) and Pathan origin (1.5%) of Pakistan [15]. Another study reported in Pakistan for screening hereditary thrombophilia confirmed the presence of FVL in 14.2% of patients [16]. Kashif *et al.,* found an association of FVL with recurrent pregnancy loss in Pakistani women [17]. Similarly, Saeed *et al.,* [18] reported a frequency of 13% of FVL mutation in patients with deep venous thrombosis compared to controls. However, a recent study negated the association of FVL with adverse pregnancy outcomes in Pakistani women [19]. Initially thought to be the mutation of Caucasians, several studies have determined the FVL mutation. The survey conducted in the North-Eastern German population shows a high prevalence of 7.12% and allele frequency of 3.56% [20]. Another study conducted on healthy children in Germany also demonstrated an increased prevalence of 12% [21]. Similarly, in a study conducted on 665 healthy Hungarians, 43 heterozygous and three homozygous (6.47%) were found [22]. European Caucasians of the Mediterranean region have shown the heterozygosity ranging from as low 0 to 0.7% in the French/ Spanish Basques population to as high as 9.5% and 13.1% in certain studies conducted on the healthy population of Italy [22]. Another large-scale study in France showed a prevalence to be 3.84% [23]. The presence of FVL was found to be rare among Japanese, Chinese, Korean, and Indonesian [24-27]. Similarly, a study from Southern Iran demonstrated 4.1% heterozygous and 0.02% of allele frequency [28]. In contrast, healthy Kuwaitis of Iranian origin revealed a prevalence of 6.42% [29]. A recent study reported 6.8% of FVL in patients with venous thromboembolism in Kashmiris [30], indicating its presence in non-Caucasians its importance in disorders associated with FVL mutation. Results from north India reported the prevalence of 3.16% and allele frequency of 1.58% [31]. Another study reported 1.9% allele frequency in India [32], whereas our study showed an allele frequency of 1.5%. In disagreement, Gupta *et al.,* showed contradictory results and found a complete absence of an FVL mutation in North Indians in a case-control study [33]. The prevalence and allele frequency reported in European Caucasian studies are higher than the Asian population due to the large sample size, different geographical conditions, and study designs. In our second part of the study, we compared three methods to find a valid, more straightforward, and cost-effective method for genotyping and screening FVL mutation in developing countries. We found tetra primer ARMS-PCR rapid, economical, and more effortless than PCR- RFLP and PCR-sequencing. Compared to PCR-RFLP, Tetra primer ARMS-PCR requires less turnaround time and cost and does not require expensive equipment and technical skills, which are essential for DNA sequencing. Furthermore, a large number of samples can be detected and interpreted simultaneously by tetra primer ARMS-PCR and PCR-RFLP compared to sequencing. Although PCR-sequencing is the gold standard method for detecting various mutations, it is more suitable for the discovery of new variants rather than for screening [34]. In our study, two genotyping methods have been compared to PCR-sequencing that remove the chances of false-positive and false-negative results. Further PCR-sequencing also excludes the occurrence of other variations in proximity to the FVL mutation, such as silent mutation A1692C, which otherwise could produce false-positive results as indicated in certain studies [35]. PCR- RFLP involves the usage of MnlI restriction enzyme, which recognizes and cuts the specific sequence [36]. Restriction enzymes are used at particular sites, and the length of the fragments determines the presence of mutation or variation [37]. Whereas tetra primer ARMS-PCR requires four primers (two inners and two outers) in the same reaction incorporating deliberate mismatches at the 3′-terminal base and position -2 from 3′-terminus. The outer primers amplify the DNA sequence, whereas inner primers detect variations [38]. In our study, the results obtained from all three methods were in 100% concordance, ascertaining the tetra primer ARMS-PCR a reliable method for screening and genotyping.

CONCLUSIONS

In conclusion, the presence of FVL mutation in our population suggests screening of FVL among selective or high-risk individuals for developing thrombophilia and pregnancy complications. Tetra primer ARMS-PCR is a

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reliable and economical method appropriate for screening FVL mutation in our setup. Further large-scale studies should be conducted to determine the association of FVL with associated disorders and obstetrical complications.

Authors Contribution

Conceptualization: FFK, YMW, AMW
Methodology: FFK, HR, RN
Formal Analysis: FFK, YMW
Writing-review and editing: FFK, YMW

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] Tsalta-Mladenov M, Levkova M, Andonova S. Factor V Leiden, Factor II, Protein C, Protein S, and Antithrombin and Ischemic Strokes in Young Adults: A Meta-Analysis. Genes. 2022 Nov; 13(11): 2081. doi: 10.3390/genes13112081.
[2] KOCAAĞA A, KILIÇ H, GÜLEÇ S. Incidence and spectrum of thrombophilia in women with recurrent pregnancy loss: a retrospective study. Eskisehir Medical Journal. 2023 Jul; 4(2): 116-20. doi: 10.48176/esmj.2023.117.
[3] Wong M, Parsi K, Myers K, De Maeseneer M, Caprini J, Cavezzi A, *et al*. Sclerotherapy of lower limb veins: Indications, contraindications and treatment strategies to prevent complications–A consensus document of the International Union of Phlebology-2023. Phlebology. 2023 May; 38(4): 205-58. doi: 10.1177/02683555231151350.
[4] Bernal S, Pelaez I, Alias L, Baena M, De Pablo-Moreno JA, Serrano LJ, *et al*. High mutational heterogeneity, and new mutations in the human coagulation factor V gene. Future perspectives for factor V deficiency using recombinant and advanced therapies. International Journal of Molecular Sciences. 2021 Sep; 22(18): 9705. doi: 10.3390/ijms22189705.
[5] Ensembl genome browser. 2021. Available at: http://www.ensembl.org/Homo_sapiens/Gene/Summary.
[6] Zhang Y, Zhang Z, Shu S, Niu W, Xie W, Wan J, *et al*. The genetics of venous thromboembolism: a systematic review of thrombophilia families. Journal of Thrombosis and Thrombolysis. 2021 Feb; 51: 359-69. doi: 10.1007/s11239-020-02203-7.
[7] Roy DC, Wang TF, Carrier M, Mallick R, Burger D, Hawken S, *et al*. Thrombophilia Gene Mutations Predict Venous Thromboembolism in Ambulatory Cancer Patients Receiving Chemotherapy. Journal of Thrombosis and Haemostasis. 2023 Aug. doi: 10.1016/j.jtha.2023.07.017.
[8] Voicu DI, Munteanu O, Gherghiceanu F, Arsene LV, Bohiltea RE, Gradinaru DM, *et al*. Maternal inherited thrombophilia and pregnancy outcomes. Experimental and Therapeutic Medicine. 2020 Sep; 20(3): 2411-4. doi:10.3892/etm.2020.8747.
[9] Kwok PY and Chen X. Detection of single nucleotide polymorphisms. Current Issues in Molecular Biology. 2003 Apr; 5(2): 43-60.
[10] de Stefano V, Chiusolo P, Paciaroni K, Leone G. Epidemiology of factor V Leiden: clinical implications. InSeminars in thrombosis and hemostasis. Copyright© 1998 by Thieme Medical Publishers, Inc. 1998 Aug; 24(4): 367-79. doi: 10.1055/s-2007-996025.
[11] Abdullah WZ, Kumaraguru S, Ghazali S, Yusoff NM. Factor V Leiden and prothrombin G20210A mutations among healthy Indians in Malaysia. Laboratory Medicine. 2010 May; 41(5): 284-7. doi: 10.1309/LM9W9L8GQPCZVAYO.
[12] Khidri FF, Waryah YM, Ali FK, Shaikh H, Ujjan ID, Waryah AM. MTHFR and F5 genetic variations have association with preeclampsia in Pakistani patients: a case control study. BMC Medical Genetics. 2019 Dec; 20(1): 1-2. doi: 10.1186/s12881-019-0905-9.
[13] Kupferminc MJ, Eldor A, Steinman N, Many A, Bar-Am A, Jaffa A, *et al*. Increased frequency of genetic thrombophilia in women with complications of pregnancy. New England Journal of Medicine. 1999 Jan; 340(1): 9-13. doi: 10.1056/NEJM199901073400102.
[14] Many A, Elad R, Yaron Y, Eldor A, Lessing JB, Kupferminc MJ. Third-trimester unexplained intrauterine fetal death is associated with inherited thrombophilia. Obstetrics & Gynecology. 2002 May; 99(5): 684-7. doi: 10.1016/S0029-7844(02)01938-5.
[15] Nasiruddin ZU, Anwar M, Ahmed S, Ayyub M, Ali W. Frequency of factor V leiden mutation. Journal of College of Physicians and Surgeons Pakistan. 2005 Jan; 15(1): 15-7.
[16] Khalid S, Sajid R, Adil S, Khurshid M. Frequency of hereditary thrombophilia: an AKUH experience. Journal of Pakistan Medical Association. 2004 Mar; 54(8): 427.
[17] Kashif S, Kashif MA, Saeed A. The association of

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DOI: https://doi.org/10.54393/pjhs.v4i09.1007

factor V leiden mutation with recurrent pregnancy loss. Journal of Pakistan Medical Association. 2015 Nov; 65(11): 1169-72.

[18] Saeed A and Kashif MA. To determine the frequency of Factor V Leiden in cases of Deep Vein Thrombosis and Healthy controls. Pakistan Journal of Medical Sciences. 2015 Sep; 31(5): 1219. doi: 10.12669/pjms.315.8088.

[19] Ali SA, Moiz B, Sheikh L. Association of Factor V Leiden G1691A and Prothrombin gene G20210A mutations with adverse pregnancy outcomes. JPMA. The Journal of the Pakistan Medical Association. 2021 Jul; 71(7): 1780-4.

[20] Schröder W, Koesling M, Wulff K, Wehnert M, Herrmann FH. Large-Scale Screening for Factor V Leiden Mutation in a North-Eastern German Population. Pathophysiology of Haemostasis and Thrombosis. 1996; 26(5): 233-6. doi: 10.1159/000217213.

[21] Aschka I, Aumann V, Bergmann F, Budde U, Eberl W, Eckhof-Donovan S, *et al*. Prevalence of factor V Leiden in children with thrombo-embolism. European Journal of Pediatrics. 1996 Dec; 155: 1009-14. doi: 10.1007/BF02532520.

[22] Jadaon MM. Epidemiology of activated protein C resistance and factor v leiden mutation in the mediterranean region. Mediterranean Journal of Hematology and Infectious Diseases. 2011 Sep; 3(1): e2011037. doi: 10.4084/mjhid.2011.037.

[23] Mazoyer E, Ripoll L, Gueguen R, Tiret L, Collet JP, dit Sollier CB, *et al*. Prevalence of factor V Leiden and prothrombin G20210A mutation in a large French population selected for nonthrombotic history: geographical and age distribution. Blood Coagulation & Fibrinolysis. 2009 Oct; 20(7): 503-10. doi: 10.1097/MBC.0b013e32832f5d7a.

[24] Hashimoto K, Shizusawa Y, Shimoya K, Ohashi K, Shimizu T, Azuma C, *et al*. The factor V Leiden mutation in Japanese couples with recurrent spontaneous abortion. Human Reproduction. 1999 Jul; 14(7): 1872-4. doi: 10.1093/humrep/14.7.1872.

[25] Jun ZJ, Ping T, Lei Y, Li L, Ming SY, Jing W. Prevalence of factor V Leiden and prothrombin G20210A mutations in Chinese patients with deep venous thrombosis and pulmonary embolism. Clinical & Laboratory Haematology. 2006 Apr; 28(2): 111-6. doi: 10.1111/j.1365-2257.2006.00757.x.

[26] Palomo I, Segovia F, Parra D, Alarcón M, Rojas E. Low prevalence of Factor V Leiden and the prothrombin G20210A mutation in a healthy population from the central-south region of Chile. Revista Brasileira de Hematologia e Hemoterapia. 2009; 31: 143-6. doi: 10.1590/S1516-84842009005000042.

[27] Pepe G, Rickards O, Vanegas OC, Brunelli T, Gori AM, Giusti B, *et al*. Prevalence of factor V Leiden mutation in non-European populations. Thrombosis and Haemostasis. 1997; 77(02): 329-31. doi: 10.1055/s-0038-1655963.

[28] Karimi M, Panahandeh Shahraki GR, Yavarian M, Afrasiabi A, Dehbozorgian J, Bordbar M, *et al*. Frequency of factor V Leiden and prothrombin polymorphism in south of Iran. Iranian Journal of Medical Sciences. 2009 Jun; 34(2): 137-40.

[29] Dashti AA and Jadaon MM. Race differences in the prevalence of the factor V Leiden mutation in Kuwaiti nationals. Molecular Biology Reports. 2011 Aug; 38: 3623-8. doi: 10.1007/s11033-010-0474-7.

[30] Shafia S, Zargar MH, Khan N, Ahmad R, Shah ZA, Asimi R. High prevalence of factor V Leiden and prothrombin G20101A mutations in Kashmiri patients with venous thromboembolism. Gene. 2018 May; 654: 1-9. doi: 10.1016/j.gene.2018.02.031.

[31] Garewal G, Das R, Varma S, Chawla Y, Prabhakar S. Heterogeneous distribution of factor V Leiden in patients from north India with venous thromboembolism. Journal of Thrombosis and Haemostasis. 2003 Jun; 1(6): 1329-30. doi: 10.1046/j.1538-7836.2003.00185.x.

[32] Das R, Garewal G, Chawla Y, DHIMAN R. Prevalence of the factor V Leiden mutation in portal and hepatic vein thrombosis. Gut. 1998 Jul; 43(1): 147. doi: 10.1136/gut.43.1.146b.

[33] Gupta N, Khan F, Tripathi M, Singh VP, Tewari S, Ramesh V, *et al*. Absence of factor V Leiden (G1691A) mutation, FII G20210A allele in coronary artery disease in North India. Indian Journal of Medical Sciences. 2003 Dec; 57(12): 535-42.

[34] Engelke DR, Hoener PA, Collins FS. Direct sequencing of enzymatically amplified human genomic DNA. Proceedings of the National Academy of Sciences. 1988 Jan; 85(2): 544-8. doi: 10.1073/pnas.85.2.544.

[35] Huber S, McMaster KJ, Voelkerding KV. Analytical evaluation of primer engineered multiplex polymerase chain reaction-Restriction fragment length polymorphism for detection of factor V Leiden and prothrombin G20210A. The Journal of Molecular Diagnostics. 2000 Aug; 2(3): 153-7. doi: 10.1016/S1525-1578(10)60631-9.

[36] Ye S, Dhillon S, Ke X, Collins AR, Day IN. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Research. 2001 Sep; 29(17): e88-88. doi: 10.1093/nar/29.17.e88.

[37] Ota M, Fukushima H, Kulski JK, Inoko H. Single nucleotide polymorphism detection by polymerase

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DOI: https://doi.org/10.54393/pjhs.v4i09.1007

chain reaction-restriction fragment length polymorphism. Nature protocols. 2007 Nov; 2(11): 2857-64. doi: 10.1038/nprot.2007.407.

[38] Thiel T, Kota R, Grosse I, Stein N, Graner A. SNP2CAPS: a SNP and INDEL analysis tool for CAPS marker development. Nucleic Acids Research. 2004 Jan; 32(1): e5-. doi: 10.1093/nar/gnh006.

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