Case-Control Study of Factor V Leiden and Prothrombin G20210A Mutations as Thrombophilia Risk Factors in Young Algerians (<45) Using LightCycler PCR.

Mechri Adem [2,3] *, Sifi Karima [1,2] , Hanachi Sabah [1,2] , Benlatreche Cherifa [1]

[1] Biochemistry laboratory, University Hospital, Constantine, Algeria

[2] Laboratory of Biology and Molecular Genetics, Faculty of Medicine, Salah Boubnider University, Constantine 3, Algeria

[3] Larbi Ben M'Hidi University, Oum El Bouaghi, Algeria

*Corresponding author email: **Mechri Adem**

Abstract

Introduction. Thrombophilia is a hereditary disorder caused by mutations affecting the coagulation system. Several genetic mutations are linked to thrombophilia, including factor V Leiden (G1691A) and prothrombin G20210A mutations. Genetic testing plays a crucial role in assessing risk factors and guiding the clinical management of individuals predisposed to hereditary thrombophilia.

Materials and Methods. This study included 200 young Algerian participants under 45, comprising 86 patients with venous thromboembolism and 114 healthy controls. All subjects (cases and controls) who had identifiable risk factors for provoked thrombosis were excluded from this study. Factor V Leiden and prothrombin G20210A mutations were detected by LightCycler PCR.

Results. Factor V Leiden mutation was more frequent (19.8%) than the prothrombin G20210A mutation (2.3%). The result demonstrate a significant positive relationship between factor V Leiden and the occurence of venous thromboembolim ($p=0.001$). The prothrombin G20210A mutation was no significantly associated with venous thromboembolism ($p > 0.05$).

Conclusion. Factor V Leiden was more prevalent among patients with VTE compared to controls, highlighting its role as a significant genetic risk factor for hereditary thrombophilia.

Keywords: Factor V Lieden, Prothrombin G20210A, Venous Thrombembolism, Hereditary Thrombophilia

Introduction

Hereditary thrombophilia is a genetic disorder characterized by a higher susceptibility to thrombotic events at young age, resulting from inherited abnormalities in the coagulation system. It involves mutations in coagulation proteins that disrupt the normal balance between coagulation and fibrinolysis, leading to an abnormal blood clot formation [Manucci PM. 2000; Khan S & Dickerman JD. 2006].

Among the most deeply studied genetic risk factors for hereditary thrombophilia is the factor V Leiden (FVL) mutation, caused by a single nucleotide substitution (G1691A) in the factor V gene. This mutation causes resistance to activated protein C, thereby increasing the risk of venous thrombosis [Rosendaal FR.1999]. Another major contributor is the prothrombin (PT) G20210A mutation, which is associated with elevated prothrombin levels and heightened tendency for clot formation [Huberfeld G, *et al*., 1998].

The identification of these genetic risk factors is crucial for achieving precise risk stratification in individuals with a personal or family predisposition to thromboembolic disorders, particularly in the context of hereditary thrombophilia [Stevens SM, *et al*. 2016].

This study aimed to evaluate the prevalence of the FVL, and PT G20210A mutations in young Algerian poplation (≤ 45 years), and explore the potential interactions among these mutations to clarify their roles in hereditary thrombophilia.

Materials and Methods

This prospective case-control study was conducted at the laboratory of Biology and Molecular Genetics, Constantine Hospital, Algeria, from 2017 to 2019. Patients were recruited from the Cardiology and Internal Medicine departments of the University Hospital of Constantine. A total of 86 patients under the age of 45, diagnosed with venous thromboembolism (VTE) using Doppler ultrasound, were included. The study excluded patients with provoked VTE, defined by obesity, pregnancy, puerperium, use of oral contraceptives, smoking, trauma, surgery, immobilization, or cancer.

The control group consisted of 114 apparently healthy adults representing the Algerian population. Individuals in this group met the inclusion criteria of being healthy, with no

family history or clinical evidence of thrombosis, a normal body mass index (BMI < 25 kg/m^2), and an age under 45 years.

Peripheral venous blood was collected in disodium EDTA, DNA was isolated from white blood cells by phenol-chloroform extraction and ethanol precipitation. Genetic testing was performed for the following mutations: FVL, and PT G20210A.

The genetic analyses for FVL and PT G20210A mutations were performed with the aid of a commercial kit using real-time PCR (Light Cycler, Roche Diagnostics GmbH, Roche Molecular Biochemicals, Mannheim, Germany)

A 222 bp fragment of the factor V gene and a 165 bp fragment of the PT gene were amplified from genomic DNA using specific primers.

The amplicon is detected by fluorescence using a pair of HybProbe Probes, which hybridize to an internal sequence of the amplified fragment during the PCR cycle. One probe is labeled at 5'-end with Lightcycler® Red 640-N-hydroxy-succimide ester (Red 640-NHS ester), the other probe is labeled at the 3'-end with fluorescein. HybProbe probes determine the genotype via melting curve analysis.

Table 1: Melting curve analysis of the three possible genotypes for the FVL and the PT G20210A mutations

Template DNA containing	Number of melting peaks	T_M of melting peaks of the FVL	T_M of melting peaks of the PT G20210A
Homozygous wild-type genotype	1	65°C ± 2.5°C	59°C ± 2.5°C
Heterozygous genotype	2	65°C ± 2.5°C 57°C ± 2.5°C	59°C ± 2.5°C 49°C ± 2.5°C
Homozygous mutant genotype	1	57°C ± 2.5°C	49°C ± 2.5°C

Figure 2 presents the melting curve profile obtained through real-time PCR analysis for the FVL and the PT G20210A mutations in selected samples from the study groups. Melting curve analysis represente the two genotypes for both mutations: homozygous wild-type genotype (GG), heterozygous genotype (GA).

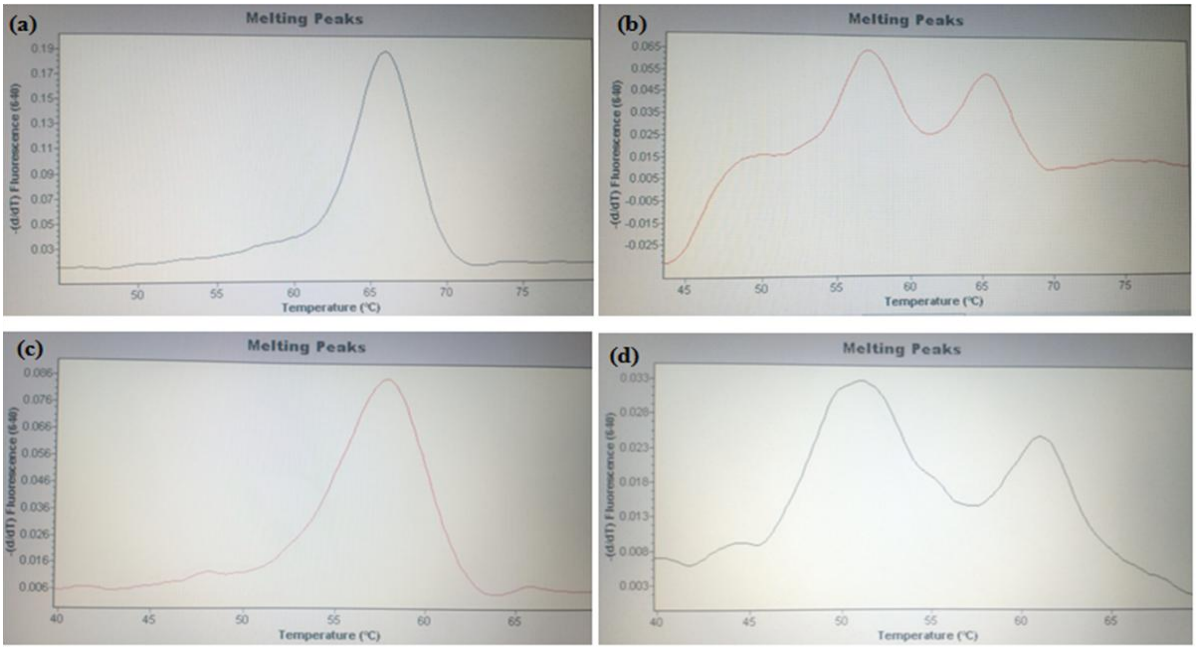

Figure 2: Melting curve analysis of FVL and PT G20210A mutations using real-time PCR. FVL: **(a)** wild type, **(b)** heterozygous genotype. PT: **(c)** wild type, (d) heterozygous genotype

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 23.0 . The chi-square test was employed to compare paired frequencies. A p-value of less than 0.05 was deemed statistically significant.

The odds ratio (OR) was also calculated to estimate the strength of association between the two categorical variables. The odds ratio represents the odds of an event occurring in case group relative to the odds of it occurring in control group.

The Student test was also calculated to compare variance for quantitaves variables

RESULTS

In our study, 200 subjects were enrolled, consisting of 86 cases and 114 controls. The demographic characteristics of cases and controls are summarized in Table 2. The case group included a higher proportion of females (62.8%), with a M/F sex ratio of 0.6, while the control group showed a similar female predominance (58.8%), with a M/F sex ratio of 0.7. No significant association was found between gender and the incidence of VTE ($p > 0.05$). The mean age of the case group was 32.53 years, while the mean age of the control group was 33.39 years. No statistically significant difference between the two groups ($p > 0.05$).

Table 2: Demographic characteristics of cases and controls

Variable	Cases (n = 86)	Controls (n = 114)
Number	86	114
Age (mean ± SD)	32.53 ± 8.48	33.39 ± 7.86
Range	15 - 45	20 - 45
Sex, n (%)		
Male	32 (37.2%)	47 (41.2%)
Female	54 (62.8%)	67 (58.8%)

n: Number

The frequencies and the prevalence of the FVL and PT G20210A mutations in patients and controls are shown in Table 3 and Table 4.

The FVL mutation, one homozygous (1.2%) and 16 (18.6%) heterozygous idividuals were found in the patient group, with no homozygous and 4 (3.5%) heterozygous idividuals were observed in the control group. A highly significant relationship was observed (X^2 13.944; p = 0.001), indicating a strong association between the different genotype of FVL mutation and VTE. The FVL mutation was more frequent in patients than in controls (10.5% vs. 1.8%) (X^2 13.789; p <0.0001). An estimated odds ratio of 6.775 was observed in patients carrying this mutation (95% CI: 2.189-20.974). On the other hand, a non-significant relationship was observed between gender and genotype, indicating that there are likely independent (X^2 2.817; p = 0.244).

Recurrence of VTE was observed in 6 patients with the FVL mutation. Among them, 1 patient (6.3%) was homozygous, and 5 patients (31.3%) were heterozygous. A significant association was found between recurrent VTE and the FVL mutation (X^2 6.835; p = 0.033).

A family history of VTE was reported in 13 patients and 4 of them (30.8%) carried the FVL mutation. A significant association was also observed between family history of VTE and the presence of the mutation (X^2 6.011; p = 0.05).

The frequency of the PT G20210A mutation was found to be higher in patients (1.2%) compared to controls (0.4%), although this difference did not reach statistical significance (X^2 0.696; p = 0.404; OR: 2.690 [IC 95%: 0.240 - 30.166]). This suggests that, while slighytly more common in patients, the mutation may not be strongly associated with disease status in this cohort.

Upon analysis of the genotypic distribution, the homozygous genotype of the PT G20210A mutation was not detected in either the patient or control groups, indicating that no individuals carrying two copies of the mutant allele were identified in the study population.

No significant association was observed between the two genotype of PT G20210A mutation and gender in patients (X^2 1.213; p = 0.271) and in controls (X^2 0.708; p = 0.4), suggesting that the mutation is distributed similarly across sex.

Additionally, no association was found between personal recurrence of VTE and the PT G20210A mutation (X^2 0.468; p = 0.494), nor between family history of VTE and the mutation (X^2 0.365; p = 0.546). These indicate that the presence of the mutation is not significantly linked to recurrence or hereditary risk within this study population.

Table 3: Frequencies of the factor V Leiden, and prothrombin G20210A genotypes in the patient group

	Genotype frequency				
	Wild, n(%)	Heterozygote, n(%)	Homozygote, n(%)	Mutation prevalence (%)	Mutation frequence (%)
Factor V Leiden	69 (80,2%)	16 (18,6%)	1 (1,2%)	19,8%	10,5%
Sex (M/F)	27/42	4/12	1/0		
Prothrombin G20210A	84 (97,7%)	2 (2,3%)	0	2,3%	1,2%
Sex (M/F)	32/52	0/2			

Table 4: Frequencies of factor V Leiden, and prothrombin G20210A genotypes in the control group

	Genotype frequency				
	Wild, n(%)	Heterozygote, n(%)	Homozygote, n(%)	Mutation prevalence (%)	Mutation frequence (%)
Factor V Leiden	110 (96,5%)	4 (3,5%)	0	3,5%	1,8%
Sex (M/F)	47/63	0/4			

Prothrombin G20210A	113 (99,1%)	1 (0,9%)	0	0,9%	0,4%
Sex (M/F)	47/66	0/1			

Discussion

This study aimed to determine whether age, gender, and specific genetic risk factors, such as the FVL and PT G20210A mutations, are associated with the occurrence of VTE.

In our study, we found a predominance of females, accounting for 54 of 86 patients (62.8%) and 67 of 114 controls (58.8%). The proportion of women in both groups is similar, with a clear predominance of females.

According to our study, Mbarek L. et *al*. (2022) conducted a study on VTE in the Tunisian population, suggesting that women were predominantly affected [Mbarek L *et al.*, 2022]. As was found in a study done by AlSheef M. *et al.* (2022) in Saudi Arabia [AlSheef M *et al.*, 2022].

It has also been observed in a study conducted by Fall O.A.T. et *al*. (2014) in an African population that VTE appears to be more prevalent in females than males, with 81 out of 105 cases (77%) and 125 out of 200 controls (62%) being female. This predominance of females in both groups was found to be statistically significant (OR = 2.21; p = 0.009) [Fall AOT *et al.*, 2014].

This overrepresentation of women might suggest a potential association between sex and the occurrence of VTE. However, in our study, the analysis shows that there is no significant association, suggesting that female sex do not affects the occurrence of a thromboembolic event ($p > 0.05$).

Our study extends previous research by focusing on selected patients with age under 45 years and without environmental factors as mentioned above, such as heredity thrombophilia testing is necessary to explain the high risks of women to have thrombosis.

According to the literature, the occurrence of VTE is more commonly observed in females, a trend that has been widely attributed to physiological and hormonal changes during reproductive period. According to Middeldorp S. et *al*. (2022), the risk of VTE during pregnancy and puerperium is estimated to be four to five times higher than in non-pregnant

women [Middeldorp S *et al.*, 2022]. Meng K. et *al*. (2014) demonstrated that women in the puerperium have a significantly increased risk of developing VTE, highlighting the need for careful monitoring and preventive strategies during this vulnerable period [Meng K *et al.*, 2014]. Khialani D. et *al*. (2020) highlighted that women with inherited thrombophilia and use hormonal contraceptive were associated with heightened risk of thrombosis, and this risk was variable and depend on the types of progestogens [Khialani D *et al.*, 2020].

Additionally to what has been previously said, in our study, the female patients were selected based on specific inclusion criteria, excluding those with no precipitating risk factors such as pregnancy, puerperium, and hormonal contraception. Therefore, the predominance of women observed in this study may be associated with other underlying factors, whether genetic or acquired. The highlights the importance of further investigating potential causes that may contribute to this predominance.

The prevalence of FVL in our study was observed in 17 patients (19.8 %) and in 3.5 % of the healthy control group. Chalal N et *al*. (2015) demonstrated that the prevalence of FVL was higher among the Algerian population with VTE [Chalal N *et al.*, 2015]. However, several studies have shown considerable variation in the prevalence of this mutation among patients with VTE, as shown in Table 5.

Table 5: Prevalence of FVL among patients with VTE in selected countries worldwide

Region	Country	Prevalence	References
North Africa	Algeria	12.5%	[Chalal N *et al.*, 2015]
	Tunisia	18%	[Mezrigui R *et al.*, 2024]
	Egypt	36.8	[Essa HH *et al.*, 2019]
Europe	France	3.84%	[Mazoyer E *et al.*, 2009]
	Poland	5.0%	[Herrmann FH *et al.*, 1997]
	North-Western Greece	16.2%	[Ioannou HV *et al.*, 2000]
	Turkish	11.3%	[Kupeli E *et al.*, 2011]
	Netherlands	2.9%	[Herrmann FH *et al.*, 1997]
	Swiss	9.0%	[Méan M *et al.*, 2017]

Middle East	Saoudi Arabia	5.9%	[Madkhaly F *et al.*, 2021]
	Liban	56.9%	[Kreidy R, 2012]
	Central Iran	17%	[Ehsani M *et al.*, 2018]
Asia	Pakistan	14.5%	[Ali N *et al.*, 2014]
	India	1.3%	[Herrmann FH *et al.*, 1997]
	China	0 %	[Jun ZJ *et al.*, 2006]
Latin America	Argentina	5.1%	[Herrmann FH *et al.*, 1997]
	Costa Rica	2.0%	[Herrmann FH *et al.*, 1997]
	Venezuela	1.6%	[Herrmann FH *et al.*, 1997]

These studies clearly demonstrate that the prevalence of FVL mutation fluctuates significantly across global populations, with high prevalence reported in Lebanese population and low prevalence in India and complet absence in the Chinese population. This marked geographic and ethnic variation likely reflects genetic backgrounds and can be attributed to genetic, historical and evolutionary factors [Herrmann FH *et al.*, 1997; Kreidy R, 2012; Ehsani M *et al.*, 2018].

Our data revealed a highly significant association between the FVL mutation and VTE, with a p-value less than 0.0001. This finding underscores the potential role of this mutation as a major genetic risk factor for VTE disease in this study population.

This result is consistent with several previous studies that have reported similarly strong associations between the FVL mutation and VTE. as illustrated by Mezrigui R. et *al*. (2024), who reported that among 50 patients, 18% were found to carry the FVL mutation, which showed a statistically significant association with the occurrence of VTE, with a p-value of 0.004 [Mezrigui R *et al.*, 2024]. Similarly, in a study conducted by Ioannou H. V. et *al*. (2000), FVL was identified in 16.2% of patients diagnosed with VTE. The presence of this mutation was found to be significantly associated with an increased risk of VTE, as demonstrated by statistical analysis, which found a p-value of less than 0.0001 [Ioannou HV *et al.*, 2000]. According to Saeed A. et *al*. (2015), the presence of FVL was significantly

associated with deep venous thrombosis, with an odds ratio of 7.32 (p = 0.003), indicating a markedly increased risk [Saeed A *et al.*, 2015].

The association between FVL and VTE has been consistently confirmed across different populations and study design. Pooled data from the literature report odds ratios ranging from 2.3 to 11.45 indicating a strong and consistent increase in VTE risk among carries of the FVL mutation [Hosseini S *et al.*, 2015; Kupeli E *et al.*, 2011; Moussaoui S *et al.*, 2017; Ali N *et al.*, 2014; Saeed A *et al.*, 2015; Takhviji V *et al.*, 2021]. These variations may reflect differences in study design, population genetics, environmental risk factors, and sample sizes.

The recurrence of VTE was observed in 6 patients (37.5%) carrying the FVL mutation. This result indicates an association between the FVL mutation and the recurrence of VTE, demonstrating that the FVL mutation confers a 3.2-fold increase risk of thrombotic recurrence (p = 0.048).

Compared to other studies, our results are consistent with previous findings regarding the association between FVL mutation and the recurrence of VTE [Saeed A *et al.*, 2015; Federici FH & Al-Modhiry H, 2019; Helley D *et al.*, 1999].

The PT G20210A mutation was identified only in its heterozygous form in both the case (2.3%) and control groups (0.9%). The absence of individuals carrying the homozygous genotype is consistent with previously published studies [Mbarek L *et al.*, 2022; Hosseini S *et al.*, 2015; Kupeli E *et al.*, 2011].

In our study the risk factor associated with carrying the PT G20210A mutation is 2.6 fold higher among patients (OR: 2.690 [IC 95% : 0.240 - 30.166]). As reported in the study conducted by Helley D. et *al.*, 1999 considered that PT G20210A mutation presente in Algerian population and considere as a risk factor of VTE [Helley D *et al.*, 1999].

In the other hand, other studies (Kupeli E. et *al*. 2011; Gurgey A. et *al*., 2001; Zalavras ChG. et *al*., 2003), have examined the relationship between PT G20210A mutation and VTE, reporting that this mutation are significant risk factors to develop VTE in Turkish and caucasian populations [Kupeli E *et al.*, 2011; Gurgey A *et al.*, 2001; Zalavras ChG *et al.*, 2003]. Also reported by Bank I et *al*. (2004) and Simone B. et *al*. (2013), they found that patients with the homozygous genotype of PT G20210A mutation have an increased risk of VTE, with an odds ratio of 6.0. They suggested that this mutation represents a moderate risk factor for VTE [Bank I *et al.*, 2004; Simone *et al.*, 2013].

In our finding, none of the patients carrying the PT mutation experienced a recurrence of VTE, suggesting that this mutation may not be associated with an increased risk of VTE recurrence. This finding was also observed in the study conducted by Mean M. et *al*. (2017), where recurrent VTE was not associated with the PT G20210A mutation in elderly patients aged ≥ 65 years [Méan M et al., 2017].

On the other hand, the association between recurrent VTE and the PT G20210A mutation was found to be statistically significant in some studies [Tosetto A et al., 1999]; however, this relationship appeared to be influenced by the duration of the recurrence period [Wang Z & Wu H, 2025].

In our study, 13 patients have a family history with VTE, 4 patients (30.5%) have the FVL mutation ($p = 0.244$), and no patients with PT G20210A mutation was found ($p > 0.05$). The relation between these mutations and a family history of VTE was not observed in the current study. Furthermore, it should be noted that previous studies have suggested a correlation between family history and VTE, indication the genetic or hereditary factors may play a significant role in an individual's predisposition to developing VTE [AlSheef M et al., 2022; Van Sluis GL et al., 2006; Segal JB et al., 2009; Al-Ansari, R.Y. et al., 2025].

Conclusion

This study found that the prevalence of FVL was higher in patients with VTE, and that it can be considered one of the most important genetic risk factors of hereditary thrombophilia. Accordingly, our results suggest that the recurrence of VTE was associated with FVL mutation and not with PT G20210A mutation. The PT G20210A mutation did not exhibit any additional or synergistic effect on the risk of VTE.

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Conflict of interest

The authors declare no conflict of interest.

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