American Journal of Health Research
2025, Vol. 13, No. 3, pp. 131-138
https://doi.org/10.11648/j.ajhr.20251303.11

Review Article

Factor V Leiden (G1691A), Prothrombin (G20210A) and MTHFR (C677T) Mutations in Yemeni Subjects Tested for Thrombophilia

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Abstract

The Factor V Leiden (G1691A), Prothrombin gene (G20210A) and MTHFR (C677T) mutations are the significant biomarkers for evaluation of tendency for venous thrombosis. The objective of our study was to assess the frequency of factor V Leiden (G1691A), Prothrombin (G20210A) and MTHFR (C677T) variants in Yemeni subjects tested for thrombophilia. *Methods*: Our study included 441 thrombophilia subjects (138 subjects for FVL (G1691A) mutation, 164 subjects for PT (G20210A) mutation and 139 for MTHFR (C677T) mutation) who were genotyped by method of SNP Genotyping Assay (FVL, PT and MTHFR variants Real Time PCR Kits), and the allele frequencies of factor V Leiden (G1691A), prothrombin (G20210A), and MTHFR (C677T) mutations were calculated. The laboratory data of patients tested were reviewed and analyzed in the Aulaqi specialized medical laboratories. *Results*: Factor V Leiden (G1691A) mutation was present in 10% of all subjects (heterozygotes: 10%, homozygotes mutant: 0%). Prothrombin (G20210A) mutation was found in 8.5% of subjects (heterozygotes: 7.3%, homozygotes mutant: 1.2%) and MTHFR (C677T) mutation in 39.5% of subjects (heterozygotes: 34.5%, homozygotes mutant: 5%). *Conclusion*: This study reports high prevalence of FVL (G1691A), PT (G20210A) and MTHFR (C677T) mutations among subjects with thrombophilia. Consequently, genotyping assay for of the three thrombotic gene mutations have a priority in the evaluation of subjects with thrombophilia, as well as in the screening for additional clinical conditions correlated with an elevated risk of thrombosis in Sana'a city-Yemen.

Keywords

Thrombophilia, Venous Thromboembolism, Factor V Leiden (G1691A), Prothrombin (G20210A), MTHFR (C677T), Mutation, Yemen

1. Introduction

Thrombophilia is abnormal blood coagulation condition leading to increased tendency to coagulation. Individuals with hypercoagulability have a higher risk of developing thrombosis, especially venous thromboembolic disorders

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Received: 08 January 2025; **Accepted:** 12 March 2025; **Published:** 24 May 2025

(VTE) such as pulmonary embolism (PE) and deep vein thrombosis (DVT). VTE is associated with considerable morbidity and mortality [1, 2]. Positive family history for VTE has been identified as a risk factor for VTE development and increases the probability of having an inherited thrombophilic defect [3-5].

The venous thrombosis risk factors can be classified into two main groups as genetic factors, including genetic susceptibility, and acquired factors, which include surgery interventions, immobilization, traumatic injuries, obesity, pregnancy, hormone treatment, heparin administration, or the use of contraceptives [6-8]. Protein C, protein S, and antithrombin III abnormalities, either quantitative or qualitative, are examples of genetic risk factors. Furthermore, there are additional hereditary thrombophilic disorders characterized by mutations in Factor V Leiden (FVL) (G1691A), prothrombin (PT) (G20210A), and methylene tetrahydrofolate reductase (MTHFR) (C677T), which have been extensively recognized as significant genetic risk factors in individuals with or without an apparent cause for developing VTE and who have a tendency to recur [8-11].

The most significant genetic risk factor for inherited VTE is the FVL (G1691A) mutation, which results in a modified variant of coagulation factor V that remains unresponsive to inactivation by activated protein C (APC), thereby precipitating a condition of hypercoagulability referred to as activated protein C resistance (APCR). The FVL polymorphism exhibits a higher prevalence in Arab and Caucasian populations, whereas it is infrequently observed in African and Asian nations [8, 12-16].

The PT (G20210A) ranks as the second most prevalent prothrombotic polymorphism. This mutation leads to a 150% increase in Prothrombin level, resulting in a significant rise in thrombin production. The PT (G20210A) variant is prevalent in southern and northern Europe, but it is very uncommon in Asian and African countries [17-20].

The MTHFR (C677T) mutation leads to a 50% reduction in enzyme activity and an increase in blood homocysteine levels, raising the risk of thromboembolic events [21-23]. According to previous study, the MTHFR (C677T) allele is more common in Europeans than in African and African American populations [24]. We aimed to investigate the prevalence of FVL (G1691A), PT (G20210A) and MTHFR (C677T) mutations in Yemeni subjects tested for thrombophilia.

2. Materials and Methods

All Yemeni subjects, (ages of participants ranged from 1 to 60 years), referred to Aulaqi specialized medical laboratories for thrombophilia assessment after an arterial stroke or VTE event, for screening due to a positive family history, the treatment of infertility or recurrent pregnancy loss or evaluation for other clinical conditions linked to an elevated risk of thrombosis from January 2018 to December 2024 were analyzed and reviewed. The study will be conduct by review the molecular tests from our Laboratory Information System for subjects who tested positive for mutations in FVL (G1691A), PT (G20210A) and MTHFR (C677T) mutations. The medical records of every participant sent to the laboratory were reviewed and gather demographic and clinical data, such as age, gender, laboratory results, and outcome. The findings for the mutations in MTHFR (C677T), PT (G20210A), and FVL (G1691A) will be compiled and presented as frequencies and percentages.

2.1. Molecular Testing

Molecular testing for FVL (G1691A), PT (G20210A), and MTHFR (C677T) mutations was performed on blood samples collected between January 2018 and December 2024. The SNP Genotyping Assay (FVL, PT and MTHFR variants Real Time PCR Kits, SNP Biotechnology R and D Ltd., Turkey) was used for molecular analysis. A system condition including wild type PCR master mix, mutant type PCR master mix and DNA control reagents were used for each variant. Using patented SNP analyses, the FVL, PT and MTHFR variants real time PCR kit offers reagents in a ready to use master mix format that has been specially optimized for 5′nuclease PCR. The test protocol was designed for use with specific primers and probe sequences. Stage (1) of the instrument will operate at 95°C for 3 minutes, followed by stage (2) at 95°C for 15 seconds and stage (3) at 60°C for 30 seconds. This cycle was repeated 30 times. Genotypic and allelic frequencies of FVL, PT and MTHFR variants will be conducted automatically.

2.2. Statistics

Genotypic frequency and percentage were performed by SPSS version 26. The Allelic frequency was analyzed using descriptive statistics (Allele Frequency online Calculator). The Hardy-Weinberg equilibrium was assessed for each variant genotypic distribution using Chi square ($\chi 2$) test (Hardy-Weinberg online calculator).

3. Results

The study included all samples whose results were obtained between January 2018 and December 2024. There were 441 subjects included 138 subjects (58 male and 80 female) for FVL (G1691A) mutation, 164 subjects (78 male and 86 female) for PT (G20210A) mutation and 139 subjects (50 male and 89 female) for MTHFR (C677T) mutation. The subjects were between the ages of one and sixty years.

The single nucleotide polymorphism (SNP) Genotyping Assay was carried out to detect the mutations and determine genotypes of subjects for the three target genes. The genotypic distributions of FVL, PT and MTHFR variants were consistent with the Hardy-Weinberg equilibrium ($P>0.05$). The prevalence of mutations, genotypes distribution and al-

lele frequencies of studied subjects were summarized in Tables 1 and 2. Prevalence of FVL (G1691A) mutation among 138 subjects was 10% (14 subjects), where all had G/A mutant genotype (heterozygote individuals). The PT (G20210A) mutation prevalence among 164 subjects was 8.5% (14 subjects) of which 1.2% (2 subjects) had the A/A mutant allele (homozygote mutant individuals) and 7.3% (12 subjects) had G/A genotype (heterozygote individuals). Meanwhile, the frequency of MTHFR (C677T) mutation was 39.5% (55 subjects), where 5% (7 subjects) and 34.5% (48 subjects) had T/T mutant allele (homozygote mutant individuals) and C/T allele (heterozygote individuals), respectively. The mutant (risk) allele frequencies were 5.1% (A allele) for FVL (G1691A) variant, 4.9% (A allele) for PT (G20210A) variant and 22.3% (T allele) for MTHFR (C677T) variant.

Table 1. *Prevalence of gene mutations.*

Type of mutation	No. of subjects (n=441)	Positive subjects (n)	Positive subjects (%)
Factor V Leiden (G1691A) (Heterozygous and Homozygous Mutant)	138	14	10
Prothrombin (G20210A) (Heterozygous and Homozygous Mutant)	164	14	8.5
MTHFR (C677T) (Heterozygous and Homozygous Mutant)	139	55	39.5

Table 2. *Genotypic and allelic frequencies observed in the studied population.*

	Factor V Leiden (G1691A), rs6025 n=138 n (%)	Prothrombin (G20210A), rs1799963 n=164 n (%)	MTHFR (C677T), rs1801133 n=139 n (%)
Genotypic frequencies			
Homozygous (Wild type)	124 (90%) (G/G)	150 (91.5%) (G/G)	84 (60.4%) (C/C)
Heterozygous	14 (10%) (G/A)	12 (7.3%) (G/A)	48 (34.5%) (C/T)
Homozygous (Mutant)	0 (0%) (A/A)	2 (1.2%) (A/A)	7 (5%) (T/T)
Allelic frequencies			
Allele 1 (Wild)	94.9% (G)	95.1% (G)	77.7% (C)
Allele 2 (Mutant)	5.1% (A)	4.9% (A)	22.3% (T)

4. Discussion

Assessment of risk genetic factors has become essential in diagnosing patients showing the signs and symptoms of venous thrombosis [25]. Deep vein thrombosis (DVT) and pulmonary embolism (PE) are two different forms of venous thromboembolism (VTE) disease. It ranks as the third most common cardiovascular condition. DVT is the most common of VTE disease [26]. The disorder known as thrombophilia, which raises the risk of thrombosis, can be acquired or genetic. It is a complex condition where the environment and genes must interact for clinical symptoms to develop. This condition is the main factor contributing venous thromboembolism (VTE) [27, 28]. The prevalence of genetic mutations in thrombophilia genes differs depending on populations and ethnic groups. Factor V Leiden (FVL), prothrombin (PT) and methylene tetrahydrofolate reductase (MTHFR) pathogenic variants are the major hereditary causes of inherited thrombophilia and thrombosis [29].

The FVL (G1691A) mutation, regarded as the most significant genetic contributor to inherited VTE, which involves a single-nucleotide polymorphism of substitution of G (guanine) to A (adenine) at nucleotide position 1691 in exon 10 of the factor V gene. This genetic alteration (FVL mutation) leads to a modified form of coagulation factor V that is resistant to inactivation by activated protein C, resulting in a state of hypercoagulability or increased susceptibility to thrombosis [30]. In the other words, FVL (G1691A) mutation causes in a single amino acid substitution, where argi-

nine is replaced by glutamine at amino acid 506 position. This mutation removes the Arg506 cutting point within Factor V for activated protein C. FVL (G1691A) variant raises the thrombosis risk as activated form of protein C (physiological anticoagulant) is unable to bind and inactivate factor V because the mutation alters the binding domain of activated protein C on factor V. Therefore, the factor V remains active, which raises the risk of thrombosis [31, 32]. The most prevalent inherited risk factor associated with venous thrombosis is the FVL (G1691A) mutation. Among individuals with thrombosis, this mutation is most common in Europeans (15%), and is found in almost 5% of white Americans and Canadians [33, 34]. In Asia, the healthy populations of Saudi Arabia and northern India had a 2.5% and 1.9% prevalence of this mutation, respectively [35].

In the clotting process, Prothrombin (PT), also known as coagulation factor II undergoes proteolytic cleavage to generate thrombin, which then transforms fibrinogen into fibrin and activates platelets. Elevated prothrombin levels and an approximately three-fold increase in the risk of venous thrombosis have been linked to the substitution of A for G at position 20210, which is in the 3/UTR region of the prothrombin gene [36]. The second most important genetic risk factor for inherited VTE is the PT (G20210A) mutation of the prothrombin gene. This mutation results in higher than normal levels of plasma prothrombin (up to 25% increase) without affecting the functional properties of the prothrombin molecule. [18, 37]. The risk of pulmonary emboli (PE) and deep vein thrombosis (DVT) is nearly doubled by this mutation [38]. Although the PT (G20210A) mutation raises the risk of deep venous thrombosis to a less than the FVL (G1691A) mutation, it presents in around 2.3% of the healthy population and approximately 6.2% of individuals with venous thrombosis [39]. The PT (20210A) variant is more prevalent among the healthy population of Southern Europe (3%), then in Northern Europe (1.7%), but it is extremely rare in Asian and African populations (0%) [20].

Methylenetetrahydrofolate reductase (MTHFR) is enzyme that catalyzes the formation of N-5-methyltetrahydrofolate from N-5,10-methylenetetrahydrofolate, which is the donor of methyl group in the methylation of homocysteine to methionine, a common mutation in MTHFR gene, MTHFR (C677T) variant, has been implicated with hyperhomocystinemia [40]. Elevated total homocysteine level has a toxic effect on the vascular endothelium and clotting cascade, it has been correlated with an increased risk of venous thrombosis [23]. The MTHFR (C677T) polymorphism mention to the substitution of cytosine (C) to thymine (T) at nucleotide position 677 in the gene of MTHFR, leading to the amino acid replacement of valine to alanine at position 222. The mutant enzyme shows reduced activity, and individuals who are MTHFR (C677T) homozygous (mutant) have a threefold increased risk of developing cardiovascular diseases prematurely (cardiovascular diseases before their time) [41]. The MTHFR (C677T) variant is highly prevalent in healthy European populations; Finland and the Netherlands have the lowest allele frequencies (25.1%) and (27.4%), respectively, in contrast, Italy exhibits the highest frequency (45%), followed by France (34%-36%), Hungary (33.7%), and Spain (33%). Compared to other ethnic groups, the prevalence of MTHFR (C677T) mutation is markedly lower in Africa and African Americans populations (3%) than in other populations [24, 42]. The prevalence of MTHFR (C677T) mutation among the vein thrombosis patients is 55.6% [43], while it is 42% in patients with thrombophilia [44].

The prevalence of thrombophilia related with pathogenic mutations in the FV, PT and MTHFR genes among Arabs has not been well or extensively studied [15]. Our study is the first to assess the prevalence of thrombophilia associated with FVL (G1691A), PT (G20210A), and MTHFR (C677T) mutations in Yemen.

In the present study involving subjects with thrombophilia, the prevalence of MTHFR (C677T) mutation was higher compared to FVL (G1691A) and PT (G20210A) mutations. The prevalence of FVL (G1691A) mutation was also higher than that of PT (G20210A) mutation which aligns with findings from studies on healthy individuals in Saudi Arabia [45], Turkish population [46] and Greek population [12], as well as in subjects with thrombophilia or thrombosis in Saudi Arabia [14], Lebanese [11], Kashmiri [8], Eastern Turkey [47] and Black Sea area of Turkey [48]. In contrast, the prevalence of these pathogenic mutations differed from our findings in Tunisian patients with cerebral venous thrombosis [49] and Iranian Patients with venous thrombosis [50], where FVL (G1691A) mutation was found to be more frequent than PT (G20210A), and MTHFR (C677T) mutations.

The present study showed that 10% of study subjects were positive for FVL (G1691A) mutation (10% heterozygous and 0% homozygous), this percentage is higher than that reported in a previous study conducted in Saudi Arabia (5.9%) in samples tested for subjects with thrombophilia [14], as well as the 6.8% frequency observed in Kashmiri patients with venous thromboembolism [8]. Our findings were consistent with a study conducted in Eastern Turkey population which found that, the prevalence of FVL (G1691A) mutation was 9.2% among patients with Venous Thromboembolism [47]. The pathogenic variant (G1691A) in the FVL gene was found less frequently in our analyzed group compared to previously published data form Lebanon [11], Tunisia [49] and Turkey [26]. Various factors, such as study group selection, criteria of exclusion, regional conditions, or ultimately the test method chosen, could contribute to discrepancies or variations in the prevalence of the examined FVL variant (G1691A) in our study compared to others.

In our study regarding the variant (G20210A) in the PT gene which is the second most significant inherited prothrombotic risk factor, we found that the frequency of this mutation was 8.5% among 164 subjects with thrombophilia. In comparison, among patients with thrombophilia in Saudi Arabia, the PT (G20210A) variant was detected at a frequency of 2.4%, while it was found in 11.8% in the Lebanese

population, 2.8% in the Kashmiri subjects, 8.9% in subjects from Iran and 4.6% in subjects from Eastern Turkey [8, 11, 47, 50]. The disparities in the prevalence of the PT (G20210A) variant between our study and others may be explained as discussed previously.

The prevalence of MTHFR (C677T) mutation observed in this study was lower than that reported in Lebanese population (83.3%), Black Sea region (Tokat) in Turkey (52.7%) and Eastern Turkey (44.8%) [11, 47, 48]. Moreover, the present study revealed that the frequency of MTHFR (C677T) mutation in the subjects with thrombophilia was 32% which exceeds the rates reported for this mutation in thrombophilia cases in Tunisia (23.1%), Iran (17.9%) and Turkey (19%) [26, 49, 50].

In our study, the distribution of the A allele (mutant or risk allele) of the FVL gene and PT gene were 5.1% and 4.9% respectively. The distribution of the T allele (mutant or risk allele) of the MTHFR gene was 22.3%. In comparison, previous study conducted on patients with thrombophilia in Tunisia found that the frequency of FVL (G1691A) A allele was 17.3%, the frequency of PT (G20210A) A allele was 0% and the frequency of MHTFR (C677T) T allele was 11.5% [49], The distribution of the risk alleles in Kashmiri patients with thrombophilia was 3.8%, 1.4% and 11.6% for the A allele of FVL (G1691A), A allele of PT (G20210A) and T allele of MTHFR (C677T) respectively [8]. Similarly, in Croatia, the distribution of the risk alleles were 8%, 2% and 32% for the A allele of FVL (G1691A), A allele of PT (G20210A) and T allele of MTHFR (C677T) respectively [51]. In Poland, the A allele frequencies were 7% and 2.7% for FVL (G1691A) and PT (G20210A) respectively) [27], while Turkey (Western Black Sea Region) had 11.7%, 3.6% and 29% for the A allele of FVL (G1691A), A allele of PT (G20210A) and T allele of MTHFR (C677T) respectively [52]. Finally, in Bosnia and Herzegovina, the mutant alleles frequencies were 10.5%, 1.4% and 33.8.% of A allele of FVL (G1691A), A allele of PT (G20210A) and T allele of MTHFR (C677T) respectively [53].

The MTHFR (C677T) mutation is found to have a higher prevalence among healthy subjects, compared to FVL (G1691A) and PT (G20210A), according to worldwide distribution rates (6-32%, 3-10% and 1-2% for MTHFR (C677T), FVL (G1691A) and PT (G20210A) mutations respectively) [45]. Studies in Tunisians and Croatians have suggested that the MTHFR (C677T) mutation is more common in healthy individuals than in those with thrombophilia [49, 51]. Therefore, the present study finds that the FVL (1691A) is more prevalent than PT (G20210A) and is considered as the most important genetic risk factor associated with thrombosis.

5. Conclusion

The prevalence of the three thrombotic gene mutations in Yemeni subjects with thrombophilia is reported for the first time in this study. In comparison to previously studied populations, Yemenis subjects tested for thrombophilia exhibit high prevalence of FVL (G1691A), PT (G20210A), and MTHFR (C677T) genetic variants. This is an important finding that should be closely monitored in regards to its clinical significance. Based on this information, we recommend that families of affected subjects to be genotyping and counseling for appropriate preventive measures.

Abbreviations

FVL	Factor V Leiden
PT	Prothrombin
MTHFR	Methylenetetrahydrofolate Reductase
VTE	Venous Thromboembolism
DVT	Deep Vein Thrombosis
PE	Pulmonary Embolism
SNP	Single Nucleotide Polymorphism

Acknowledgments

The authors are grateful acknowledge the DNA testing unit staff in Aulaqi specialized medical laboratories for their support and help in this study

Author Contributions

Mohammed Ahamed Hajar: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

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Conflicts of Interest

The authors declare no conflicts of interest.

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