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Prevalence of thrombophilia-associated mutations and their clinical significance in a large cohort of Lebanese patients

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ABSTRACT

Background: Mutations in the *Factor V*, *Factor II* and *MTHFR* genes are very high in the MENA region and have not been linked widely to specific entities in the hypercoagulation spectrum. In addition, the clinical effect of *MTHFR* alterations remains debatable.

Aims: Determining the frequency of mutations in Lebanese patients referred for thrombophilia testing following a thrombotic event or for familial screening and investigating for the presence of a correlation with specific clinical events, location of thrombosis and recurrence rates.

Methods: Laboratory results of Lebanese patients referred to the American University of Beirut Medical Center for thrombophilia testing from Jsanuary 2007 until December 2016 were reviewed. Molecular testing for *Factor V* (*Factor V Leiden, G1691A*), *Factor II (G20210A)* and *MTHFR (C677T)* in our CAP-accredited laboratory is based on the Vienna Lab (Viennalab diagnostics GmbH, Vienna, Austria) FV/PTH/MTHFR Strip Assay. Clinical information including age, gender, reason for testing, risk factors and history of recurrence were collected from the Electronic Health Records.

Results: For the 2002 obtained cases, the male: female ratio was 1:2 (male = 32.7%, female = 67.3%). The age ranged from 4 days to 91 years with a median of 34 years. A *G1691A* mutation of *Factor V* was present in 36.5% of all patients (heterozygotes: 31.4%, homozygotes: 5.1%). *Factor II (G20210A)* was found in 11.8% (heterozygotes: 11.4%, homozygotes: 0.4%) and *MTHFR (C677T)* in 83.3% (heterozygotes: 62.4%, homozygotes: 20.9%). Double mutations were present in 674 cases (33.6%) and triple mutations in 41 cases (2%). Clinical data could be retrieved for 1000 patients, among which 353 (35.3%) presented for workup of infertility/recurrent pregnancy problems, 271 (27.1%) following a venous thromboembolism event (DVT and/or PE), 181 (18.1%) after an arterial stroke and 22.1% (n = 221) for familial screening. Statistically significant correlations were found between the presence of a *Factor V* or *Factor II* mutation and presentation for a venous thromboembolism (VTE) or recurrent pregnancy loss. No relation was found between the different types of prothrombotic mutations and VTE location or recurrence rates.

Conclusion: This is the first study in the MENA region to evaluate the frequency of all gain of function thrombophilia genes in patients with a personal or familial history of thrombosis. The Lebanese population carries the highest rates of prothrombotic mutations worldwide. This has major implications on the application of proper screening and anticoagulation prophylaxis strategies in our region.

1. Introduction

Globally, one in four deaths occurs due to consequences of thrombosis. Occurring in more than 900,000 individuals per year in the United States alone, thrombosis constitutes one of the leading causes of morbidity and death worldwide. Thrombophilia, also called hypercoagulable or prothrombotic state, is defined as a disease marked by blood hypercoagulability. Manifestations include venous thromboembolism (VTE) including deep vein thrombosis (DVT) and subsequent pulmonary embolism (PE), arterial strokes and conception/infertility problems

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including recurrent miscarriages and severe preeclampsia (Mitchell et al., 2007). Thrombophilia can be acquired or hereditary. Acquired causes include antiphospholipid syndrome, surgery, prolonged immobilization, trauma, cancer, pregnancy and drugs such as oral contraceptives or heparin use (Goldhaber, 2010; Ocak et al., 2013). Hereditary thrombophilia originates from loss of function (mutations in antithrombin, protein C and S) or gain of function genetic alterations affecting *Factor V (factor V Leiden, G1691A)*, *Factor II (G20210A)* and *MTHFR (C677T)* (Dahlbäck and Hildebrand, 1994).

Prevalence of the above-mentioned mutations differ according to ethnicity. For example, Middle Eastern populations have been shown to harbor one of the highest frequencies of Factor V Leiden worldwide (Irani-Hakime et al., 2000a; Eid and Rihani, 2004; Kfoury et al., 2009). Mutations in the prothrombin and *MTHFR* gene also show different distribution across populations (Eid and Rihani, 2004; Kfoury et al., 2009; Jarjour et al., 2017).

Although Factor V Leiden and prothrombin mutations were proven to increase the thrombotic risk, their presence have not yet been associated with specific thrombotic events; furthermore, the role of *MTFHR* in thrombosis is still controversial (Hotoleanu, 2017). In our study, we aim at determining the prevalence of *Factor V Leiden (G1691A)*, *Factor II (G20210A)* and *MTHFR (C677T)* in a cohort of thrombophilia positive Lebanese patients who presented to our tertiary care center for evaluation after a thrombotic event or for screening purposes during the management of infertility or the presence of a positive family history. The reason for referral is determined in the aim of finding a correlation with the presence of specific mutations. Because clot formation is a complex mechanism involving several risk factors, we also shed light on underlying risk factors exacerbating the present hypercoagulability due to genetic thrombophilia.

2. Materials and methods

All Lebanese patients (both pediatric and adults; no age limit applied) referred to our tertiary care center (American University of Beirut Medical Center) for thrombophilia testing following a VTE or arterial stroke event, for screening in the presence of a positive family history or the management of infertility/recurrent pregnancy loss from January 2007 to December 2016, were reviewed. Information were retrieved for patients who tested positive for a *Factor V*, *Factor II* or *MTHFR* mutation, from our Laboratory Information System (LIS). Clinical information including age, gender, reason for testing, risk factors (obesity, previous surgery, immobility, inherited diseases, etc) and history of recurrence were collected from the Electronic Health Records (EHR). No exclusion criteria were applied.

2.1. Molecular testing

Molecular testing for *Factor V (factor V Leiden, G1691A)*, *Factor II (G20210A)* and *MTHFR (C677T)* in our CAP-accredited laboratory is based on the Vienna Lab (Viennalab diagnostics GmbH, Vienna, Austria) FV/PTH/MTHFR Strip Assay. The strip assay is based on polymerase chain reaction (PCR) and reverse hybridization principle and includes 3 successive steps. In the first step, DNA is isolated from anticoagulated blood using a Lysis Solution and GenX Tract Resin X. Then *Factor V*, *PTH*, and *MTHFR* gene sequences are simultaneously amplified in vitro, using ThermoFisher Hybaid Px2 (Massachusetts, USA) and Agilent technologies SureCycler 8800 (California, USA) thermocyclers, and biotin-labeled in a single ("multiplex") amplification reaction. Finally, the amplification products are selectively hybridized to a test strip, which contains oligonucleotide probes (wild type and mutant-specific) immobilized as parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates.

2.2. Statistical analysis

Statistical analysis was performed using SPSS statistics version 23. Chi square analysis and Fischer's exact test were performed.

2.3. Ethical considerations

This study received an approval from the Institutional Review Board at the American University of Beirut.

3. Results

Mutational analysis results retrieved from our LIS accounted for 2002 patients. Demographical data (age and gender) was available for all patients. Reason for testing, risk factors associated with the thrombotic event and presence of recurrence could be obtained for 1000 patients.

Overall, the male: female ratio was 1:2 (male = 32.7%, female = 67.3%). The age ranged from 4 days to 91 years with a mean of 36 years (median = 34 years) and a peak observed at 28 years.

A *G1691A* mutation of *Factor V* was present in 36.5% of all patients (heterozygotes: 31.4%, homozygotes: 5.1%). *Factor II (G20210A)* mutation was found in 11.8% (heterozygotes: 11.4%, homozygotes: 0.4%) and *MTHFR (C677T)* in 83.3% (heterozygotes: 62.4%, homozygotes: 20.9%). Double mutations were present in 674 cases (33.6%) and triple mutations in 41 cases (2%). Frequencies of cases with concomitant mutations in *Factor V*, *Factor II* and/or *MTFHR* are shown in Table 1.

3.1. Mutations and clinical categories

Among the 1000 patients for which clinical information could be obtained, 353 patients (35.3%) presented for workup of infertility/recurrent pregnancy problems. 271 patients (27.1%) were tested for thrombophilia genes following a venous thromboembolism event (DVT

Table 1
Frequency of double and triple gain of function thrombophilic mutations.

Mutations/Genotpye	Frequency % (number) Total $n = 2002$
Double mutations	
Factor V heterozygote, Factor II heterozygote	3 (60)
Factor V heterozygote, Factor II homozygote	0 (0)
Factor V homozygote, Factor II heterozygote	0.4 (9)
Factor V homozygote, Factor II homozygote	0 (0)
Factor V heterozygote, MTHFR heterozygote	14.7 (294)
Factor V heterozygote, MTHFR homozygote	4.6 (93)
Factor V homozygote, MTHFR heterozygote	2.3 (46)
Factor V homozygote, MTHFR homozygote	0.75 (15)
Factor II heterozygote, MTFHR heterozygote	5.3 (107)
Factor II heterozygote, MTFHR homozygote	2.2 (43)
Factor II homozygote, MTFHR heterozygote	0.3 (6)
Factor II homozygote, MTFHR homozygote	0.05 (1)
Triple mutations	
Factor V heterozygote, Factor II heterozygote, MTHFR heterozygote	1.2 (24)
Factor V heterozygote, Factor II heterozygote, MTHFR homozygote	0.5 (10)
Factor V heterozygote, Factor II homozygote, MTHFR heterozygote	0 (0)
Factor V heterozygote, Factor II homozygote, MTHFR homozygote	0 (0)
Factor V homozygote, Factor II heterozygote, MTHFR heterozygote	0.15 (3)
Factor V homozygote, Factor II heterozygote, MTHFR homozygote	0.2 (4)
Factor V homozygote, Factor II homozygote, MTHFR homozygote	0 (0)
Factor V homozygote, Factor II homozygote, MTHFR homozygote	0 (0)

and/or PE) and 181 (18.1%) following an arterial stroke. 22.1% (n = 221) of patients were referred to our lab for screening purposes due to the presence of a positive family history. Details of demographics and reason for testing can be seen in Tables 2 and 3.

An association was found between the presence of a *Factor V* mutation and referral for both infertility management ($p < 0.0001$) and VTE ($p < 0.0001$). However, no relation was found with testing after an arterial stroke ($p = 0.345$). Similarly, a relation was observed between the presence of a *Factor II* mutation and presentation for recurrent pregnancy loss ($p = 0.016$) and VTE ($p = 0.03$) but not arterial thrombosis ($p = 0.538$). Regarding *MTHFR* mutations, no association was found with any clinical presentation ($p > 0.05$).

3.2. VTE and Arterial Thrombosis Location and association with prothrombotic mutations

VTE cases consisted of pulmonary embolism in 14% (35/251 cases). Remaining cases were divided into deep vein thrombosis of the upper or lower extremities (133/251; 53%), superficial phlebitis in the upper or lower extremities (26/251; 10%), cerebral (13/251; 5%) -most commonly sinus thrombosis-and other uncommon locations (portal 17/251; 6.7%, IVC 4/251; 1.6%, renal vein 3/251; 1.2%, splenic 3/251; 1.2%, ovarian 2/251; 0.8%, retinal 8/251; 3% and mesenteric 7/251; 2.8%). Lower limb deep vein thrombosis was more likely to occur on the left side (64%) and affect proximal veins (iliac, femoral and popliteal) (74%) rather than distal veins, Table 4.

The majority of arterial thrombosis cases consisted of ischemic strokes (102/166; 61%), most commonly affecting the middle cerebral artery. Peripheral vascular occlusions occurred in 14% (24/166) of patients. Arterial occlusion of the retinal, ischemic priapism, atrial thrombus, superior mesenteric, splenic, renal, carotid in 3, 2, 24, 5, 1, 4 and 1 cases respectively.

Comparison of different mutation types with VTE and arterial thrombosis site (location and side) did not yield any statistically significant correlation ($p > 0.05$).

3.3. Prothrombotic risk factors

Risk factors for a hypercoagulable state were present in 16 out of 1000 patients. The majority (9/16; 56%) consisted of active cancer at the time of the thrombotic event (including acute lymphoid leukemia, B cell lymphoma, Ewing sarcoma, ovarian, colon and breast cancer). Two patients (12.5%) had active sickle cell disease at the time of the thrombotic event. Other risk factors included morbid obesity (3/16; 19%), hip surgery (1/16; 6.25%) and central line insertion complicated by a thrombus in the IVC (1/16; 6.25%).

3.4. Recurrence rates

The recurrence rate for patients positive for a *Factor V* mutation, a *Factor II* or an *MTHFR* mutation was 3.9%, 3.9% and 4.7%, respectively. No statistical correlation was found between the type of mutation and the presence of recurrence ($p > 0.05$).

4. Discussion

Male gender is known to be a risk factor for thrombosis. In fact, men have been shown to experience a higher number of VTE events and develop worse outcomes both in the setting of a hereditary thrombophilia and cancer associated prothrombotic states (Riondino et al., 2017; Tzoran et al., 2017). Our patients' cohort however shows a higher ratio of females to males (ratio: M: F = 1:2). To date, studies targeting healthy Lebanese adults, showed no difference in the prevalence of *Factor V*, *Factor II* and *MTHFR* mutations between males and females (Irani-Hakime et al., 2000b; Sabbagh et al., 2009; Almawi et al., 2004). However, a definite female predominance is present among Lebanese patients with a previous history of thrombotic events (Kreidy and Irani-Hakime, 2009; Kreidy et al., 2012). Adding to this, in a previous study done in our institution, most referrals for thrombophilia testing were found to originate from the obstetrics and gynecology department (Hoteit et al., 2012a) which might account for the female predominance in our cohort. Indeed, the exclusion of patients referred for infertility/recurrent pregnancy loss investigation in our cohort yields a slight male predominance (M: F = 1.2: 1). Guidelines for thrombophilia testing state that the search for an underlying genetic factor for hypercoagulability should not be performed after the age of 60 years (Pernod et al., 2009). Our cohort's age range, with 8.6% of patients being above age 60 and a maximum of 91 years, shows a potential overuse of genetic testing.

MTHFR was the most prevalent mutation in our study group, present in up to 83.3% of patients. *MTHFR* single nucleotide polymorphisms (SNPs) seem to follow a North-South incidence gradient in Europe and North America and a West-East gradient in Asia, where higher rates are present in the Middle East as compared to India and East Asia (Ameen et al., 2005). Indeed, the frequency of *MTHFR* mutations *(C677T and A1298C)* in healthy Lebanese and Syrian populations has been found to be among the highest worldwide (Jarjour et al., 2017; Almawi et al., 2004; Sabbagh et al., 2008).

Studies show that higher levels of homocysteine are present in patients with C677T homozygosity but not A1298C polymorphisms (Mahfouz et al., 2012). Increased homocysteine levels are known to increase the risk of cardiovascular diseases, strokes and recurrent pregnancy loss (Ueland and Clarke, 2007; Sadewa et al., 2002). However, major debate exists in the literature about the association between *MTHFR* and the occurrence of a thrombotic event. Some studies show a higher risk of VTE events and recurrent pregnancy loss in the presence of an *MTHFR* mutation (Den Heijer et al., 2005; Cao et al., 2013). A recent study done to determine the frequency of thrombophilia genes in Bosnian women showed an increased risk of abortions in the setting of a mutated *MTHFR* (Jusic et al., 2018). However, in a study done in Brazil comparing the prevalence of *MTHFR* mutations in fertile and infertile woman no difference was found in the frequency, even when comparing rates of homozygosity (Soligo et al., 2017). Current guidelines, including the American College of Medical Genetics and the American College of Obstetricians and Gynecologists, do not recommend *MTHFR* testing as a part of the traditional testing for thrombophilia (Hickey et al., 2013; American College of Obstetricians and Gynecologists Women's Health Care Physicians, 2013). Recent reviews also show no added benefit of

Table 2
Demographics and reason for testing for individual mutations.

			Reason for testing (n; %)			
Mutation	Age in years (median; range)	Gender (%males)	VTE	Arterial stroke	Infertility	Familial screening
Factor V Leiden (heterozygote); $n = 301$	33 (0.01–82)	40.9%	114; 38%	48; 16%	69; 23%	83; 28%
Factor V Leiden (homozygote); $n = 58$	29.5 (0.16–78)	48.3%	24; 41.3%	8; 13.8%	11; 19%	17; 29.3%
Factor II mutation (heterozygote); $n = 107$	35 (0.04–91)	43.2%	42; 40.2%	15; 14%	26; 24.3%	27; 25.2%
Factor II mutation (homozygote); $n = 6$	31.5 (28–48)	33.3%	2; 33%	1; 17%	2; 33%	1; 17%
MTHFR mutation (heterozygote); $n = 633$	33 (0.01–91)	33.5%	160; 25%	119; 19%	240; 38%	128; 20%
MTHFR mutation (homozygote); $n = 215$	35 (0.16 to 74)	31.2%	55; 25.6%	33; 15.3%	76; 35.3%	55; 25.6%

N·B: Several patients carry multiple types of thrombotic events.

Table 3
Mutation profile of thrombotic events.

			Mutations (n; %)					
Reason for testing	Age in years (median; range)	Gender (% males)	Factor V (single)	Factor V (double)	Factor II (single)	Factor II (double)	MTHFR (single)	MTHFR (double)
Infertility; $n = 350$	32 (19–57)	0%	69; 19.7%	11; 3.1%	26; 7.4%	2; 0.6%	240; 68.6%	76; 21.7%
VTE; $n = 280$	42 (0.04–91)	57.1%	114; 40.7%	25; 8.9%	43; 15.4%	2; 0.7%	160; 57.1%	55; 19.6%
Arterial; $n = 171$	41 (0.01–91)	62.6%	48; 28.1%	2; 4.7%	15; 8.8%	2; 1.2%	119; 69.6%	33; 19.3%
Familial screening; $n = 223$	28 (0.03–70)	43.5%	83; 37.2%	17; 7.6%	27; 12.1%	1; 0.4%	128; 57.4%	55; 24.7%

Table 4
Location of VTE.

Location	Frequency % (number) Total $n = 251$ VTE cases
Deep proximal LE	19.5 (49)
Deep proximal UE	4 (10)
LE, NOS	21.5 (54)
UE, NOS	0.8 (2)
Portal	6.7 (17)
Cerebral	5.2 (13)
PE	13.9 (35)
Renal	1.2 (3)
Splenic	1.2 (3)
IVC	1.6 (4)
Ovarian	0.8 (2)
Deep distal LE	6.8 (17)
Deep distal UE	0.4 (1)
Superficial proximal LE	8.4 (21)
Superficial proximal UE	2 (5)
Retinal	3.2 (8)
Mesenteric	2.8 (7)

MTHFR testing in the setting of thrombosis (Levin and Varga, 2016). In view of these data, although our study shows a higher prevalence of *MTHFR* mutations in thrombosis patients as compared to healthy Lebanese subjects (Sabbagh et al., 2008), the clinical relevance of the presence of such mutations should be further evaluated.

Similar to *MTHFR* mutations, the Mediterranean population has been found to carry the highest prevalence rates of *Factor V Leiden* worldwide (Ameen et al., 2005). Healthy Lebanese also seem to harbor the highest rates among neighboring countries. In a study done by Irane et al., *Factor V* mutations were present in 14.4% of healthy Lebanese, compared to 13.6%, 13.4% and 12.3% of Syrians, Greeks and Jordanians respectively (Irani-Hakime et al., 2000a). Almawi et al. confirmed this finding by comparing the prevalence of *Factor V* mutant alleles in Lebanon (0.0788), Tunisia (0.0351), Bahrain (0.0155) and Saudi Arabia (0.0101) (Almawi et al., 2005).

The prevalence of *Factor V Leiden* was found to be higher in our cohort (36.5% of all patients; heterozygotes: 31.4% and homozygotes: 5.1%) as compared to the healthy Lebanese population (14.4%) (Irani-Hakime et al., 2000a) and a correlation was obtained between the presence of a *Factor V* mutation and presentation for VTE and recurrent pregnancy loss, but not arterial thrombosis. Single point mutations of the *Factor V* gene lead to the transcription of a resistant protein that cannot be cleaved by activated Protein C leading to Factor V hyperactivity with subsequent activation of Factor Xa and increased prothrombin conversion to thrombin (Bertina et al., 1994). Increased thrombin levels result in a hypercoagulability profile with an increased risk of VTE events (Kujovich, 2010). Indeed, a *Factor V* mutation was found to be present in 25% of patients with a history of VTE and 50% of patients with a positive family history of a thrombotic event (Ridker et al., 1995; Rosendaal et al., 1995).

Also, the hypercoagulability state favored by the presence of a *Factor V* mutation has been shown to be associated with recurrent pregnancy loss (Kujovich, 2010). In the above-mentioned study, Bosnian infertile women were found to carry a higher rate of *Factor V* mutations (Jusic et al., 2018). Detection of *Factor V Leiden* by polymerase chain reaction and ARMS technique by Kashif et al., showed a positivity rate of 5.4% in the recurrent pregnancy loss group with a complete absence of the mutation in the control group ($p = 0.001$) (Kashif et al., 2015). In a meta-analysis by Rey et al. combining 31 studies, a strong correlation was found between activated protein C resistance and early recurrent pregnancy loss (OR = 3.48) (Rey et al., 2003).

The relationship between hereditary activated protein C resistance and the development of arterial strokes and myocardial infarctions is still not fully elucidated. In an attempt to elucidate the pathogenesis of cerebral venous and arterial thrombosis, Beye et al. found an association between the presence of activated protein C resistance and the development of CVT and arterial strokes (OR = 3.89 and 4.16) (Beye and Pindur, 2017). However, testing for the presence of a *Factor V* SNP in the setting of arterial ischemic events was proven to be beneficial only in younger patients (age < 45 years) (Kim and Becker, 2003; Soare and Popa, 2010).

The prevalence of *Factor II* mutations differs according to geographical locations, with the highest rates being present the Mediterranean population (Ameen et al., 2005). Slightly lower frequencies are found in Europe with Southern countries carrying approximately twice the frequency found in the North (Rosendaal et al., 1998). This mutation is rare in individuals coming from Asian, African, American and Australian descent (Rosendaal et al., 1998). The healthy Lebanese population was found to carry a *Factor II (G20210A)* mutation in 1–3% (Sabbagh et al., 2009; Ameen et al., 2005; Almawi et al., 2005; Tamim et al., 2002). G/G is the most common phenotype, found in 98.54%, followed by G/A in 1.46% and A/A genotypes in 0% (Sabbagh et al., 2009).

The *G20210A* mutation in the *Factor II* gene (chromosome 11) causes a transition of the G nucleotide to A at the position 20,210 in the 3′-untranslated region (Poort et al., 1996). This transition was found to be associated with higher plasma prothrombin levels (133%) and a two-fold increase in VTE risk (Poort et al., 1996). Similar to our results, the prevalence of this mutation is higher in VTE than healthy individuals (Jadaon, 2011).

In a case-control study by Rey et al., an increased risk of early recurrent pregnancy loss, pregnancy loss before 25 weeks and non-recurrent pregnancy loss was found in the setting of a *Factor II* SNP (Rey et al., 2003). In their meta-analysis, Gao et al. found an association with recurrent pregnancy loss, more specifically in European women more than 29 years of age (Gao and Tao, 2015). The relationship however is still not definite. In a study done including 4000 low-risk women, no link was found between pregnancy complications and inherited *Factor II* mutations (Silver et al., 2010). The use of heparin thromboprophylaxis in pregnant women with inherited thrombophilia due to a prothrombin mutation also showed no difference in the number of live births (Warren et al., 2009). In view of these data, testing for a *Factor II (G20210A)* mutation as part of an infertility workup is still debatable and should be decided on a case by case basis (Hotoleanu, 2017).

In a study done by Kim et al., only a weak association was found between arterial thrombotic events and *Factor II* SNPs (OR = 1.32; 95% CI, 1.03–1.69). Furthermore, this weak link was observed with myocardial infarctions and ischemic strokes but not peripheral vascular

disease (Kim and Becker, 2003). As such, further data is required to establish a causative effect of increased prothrombin on arterial thrombosis.

The prevalence of triple thrombotic mutations in our cohort was 2%, concordant with a previous study done on 2248 cases in Lebanon reporting a triple positivity rate of 1.1%, which is one of the highest rates worldwide (Hoteit et al., 2012b).

Venous thrombosis (VT) usually occurs in the deep veins of the extremities and has a potential to migrate to pulmonary arteries causing pulmonary embolism. However, in approximately 4% of cases, a thrombus forms in atypical locations such as the splanchnic (portal, hepatic, splenic and mesenteric), renal, gonadal (testicular and ovarian) and cerebral regions (Janczak et al., 2018). The occurrence of VT in atypical locations is favored by the presence of an underlying pathology in adjacent or draining organs (Tafur et al., 2011; Wysokinski et al., 2008; Brown and Cunningham, 2018). The higher percentage of atypically located VT in our cohort might be explained by the presence of several patients with an underlying malignancy or the increased tendency by clinicians to explore for an underlying hereditary thrombophilia in the setting of an atypical VT.

Current testing guidelines state that thrombophilia testing should not be performed following a provoked thrombotic event (Stevens et al., 2016) as the recurrence rate and anticoagulation risk/benefit following a provoked VTE did not differ between patients carrying a thrombotic mutation and those without a hereditary thrombophilia (Coppens et al., 2008). Thus, the discovery of a thrombotic gene mutation in the setting of a provoked event might lead to unnecessary prolongation of anticoagulation therapy and increased risk of bleeding (Hicks et al., 2013). Indeed, a risk factor was found in only 1.6% of our tested patients indicating that thrombophilia testing following a provoked thrombotic event is not systematic and being tailored to individual cases.

In our study, the association between specific mutation types, recurrence rate and VTE location could not be established. Higher recurrence rates are known to occur with unprovoked VTE, male gender and a positive family history; while effect of the presence of hereditary thrombophilia is negligible (Kyrle and Eischer, 2013; Sundquist et al., 2015). Large systematic studies show no difference in the relative risks of recurrence between *FV Leiden* and *prothrombin G20210A* positive patients (Kovac et al., 2008; Ho et al., 2006).

Similarly, a causative link between the type of prothrombotic mutation and VTE location has not yet been established. Previous studies failed at establishing a statistically significant association when comparing outcomes of *Factor V Leiden* to *Factor II* inherited thrombophilia (Kovac et al., 2010; Emmerich et al., 2001; de Moerloose et al., 2000; Margaglione et al., 2000).

5. Conclusion

This is the first study in the MENA region to evaluate the frequency of all gain of function thrombophilia genes in patients with a personal or familial history of thrombosis. The Lebanese population carries the highest rates of *Factor V Leiden*, *Factor II (G20210A)*, *MTHFR* and triple prothrombotic mutations both in healthy and affected individuals. In light of this information, adequate thrombophilia screening strategies should be applied in our region, along with careful interpretation of results, including the decision to initiate long-term anticoagulation prophylaxis. Further studies are required to fully elucidate the clinical effects of the different prothrombotic mutations, specifically *MTHFR* mutations.

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Authors' contributions

Dr. Younis and Dr. Zarif were involved in the major literature search and write-up of manuscript. Dr. Mahfouz edited the final version of the manuscript.

Declaration of Competing Interest

The authors do not have any conflict of interest to declare.

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