A Case Control Study on the Contribution of Factor V-Leiden, Prothrombin G20210A, and MTHFR C677T Mutations to the Genetic Susceptibility of Deep Venous Thrombosis

Wassim Y. Almawi,1 Hala Tamim,2 Raghid Kreidy,3 Georgina Timson,1 Elias Rahal,3 Malak Nabulsi,4 Ramzi R. Finan,3 Noha Irani-Hakime3
1Al-Jawhara Center for Molecular Medicine, Genetics, and Inherited Diseases, Arabian Gulf University, Manama, Bahrain; 2Faculty of Health Sciences, American University of Beirut; 3St. Georges University Hospital, Beirut; 4Islamic Hospital, Tripoli, Lebanon

Abstract. Background: Insofar as the inherited prothrombotic single nucleotide polymorphisms (SNPs) factor V G1691A (FV-Leiden), prothrombin (PRT) G20210A, and methylenetetrahydrofolate reductase (MTHFR), C677T are inherited risk factors of venous thromboembolism (VTE), the aim of this study was to determine the prevalence of single and combined SNPs in 198 patients with documented deep venous thrombosis (DVT), and 697 control subjects, and to estimate the associated risks.

Methods: Factor V-Leiden, PRT G20210A, and MTHFR C677T were analyzed by PCR and restriction fragment length polymorphism (RFLP).

Results: The prevalence of the heterozygote and homozygous variants for FV-Leiden (52.02 vs. 14.78%, RR 6.28), PRT G20210A (19.2 vs. 3.6%; RR 6.38), and to a lesser extent the T/T genotype of MTHFR C677T (20.71 vs. 11.0%; RR 1.49) were higher among DVT patients vs. controls, respectively. Two or more SNPs were detected in 90 of 198 patients (45.5%) and in 60 of 697 controls (8.6%), with odds ratios of 16.754 for joint occurrence of FV-Leiden and PRT G20210A, 10.471 for FV-Leiden and MTHFR C677T, and 6.283 for PRT G20210A SNPs and MTHFR 677T/T. Logistic regression analysis showed a further increased odds for FV-Leiden in combination with PRT G20210A (85.198) or homozygous MTHFR C677T (81.133), and to a lesser extent for PRT G20210A in combination with homozygous MTHFR C677T (20.812).

Conclusions: This indicates that FV-Leiden and PRT G20210A, more than MTHFR C677T, are important risk factors for DVT, and that the presence of more than one prothrombotic SNPs was associated with a significant risk of DVT.

Key Words. Venous Thrombosis; Factor V-Leiden, Prothrombin G20210A; MTHFR C677T; PCR

Introduction

Venous thromboembolism (VTE) is a multi-factorial disease, resulting from the interaction of genetic and environmental factors [1]. Among the inherited risk factors are single nucleotide polymorphisms (SNPs) in the genes coding for blood coagulation factors which induce either the synthesis of a defective protein, or the enhanced production of a procoagulant protein, and hence precipitate VTE events. The former mechanism is exemplified by the factor V gene G1691A SNP (FV-Leiden) which renders factor V resistant to activated protein C (APC) degradation [2]. The latter is exemplified by the prothrombin/factor II (PRT) G20210A, a SNP in the 3′-untranslated region of the PRT gene, which alters PRT mRNA stability, resulting in higher PRT levels. Both FV-Leiden and PRT G20210A SNPs are associated with heightened risk of VTE [3,4], and are relatively frequent among white/Caucasian populations [5], with a founder effect being suggested [6].

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, and plays a critical role in the homocysteine-methionine methylation. A reduction in MTHFR level or activity leads to hyperhomocysteinemia, characterized by increased plasma total homocysteine (Hcy) levels, and is often seen in patients with vascular diseases [7]. The C677T SNP in the MTHFR gene (A223V) results in a thermolabile enzyme and reduced enzymatic activity, and homozygotes (677 T/T) for this SNP were associated with a 50% reduction in the MTHFR activity. Whereas FV-Leiden or PRT G20210A polymorphism were considered risk factors for deep venous thrombosis (DVT), similar association between MTHFR C677T SNP and heightened risk of DVT was
controversial, with some reports suggesting an association \[8,9\], while others indicated a weak \[10\] or no association \[10,11\] of MTHFR C677T with DVT. Furthermore, it was suggested that DVT was associated with hyperhomocysteinemia, independent of MTHFR C677T \[12–14\].

Coinheritance of multiple genetic defects was significantly associated with increased risk of thrombosis \[7\], and the simultaneous occurrence of hereditary thrombophilias and/or prothrombotic polymorphisms was shown to substantially increase the risk of VTE \[14–16\]. This was highlighted by the findings that the co-presence FV-Leiden with PRT G20210A \[17,18\] increased the predicted risk of thrombotic events, since the odd ratios increased from 4.9 for FV-Leiden and 3.8 for PRT G20210A, to 20.0 for double heterozygotes \[18\]. Similarly, an increased risk of thrombosis was reported for FV-Leiden and hyperhomocysteinemia, without necessarily involving the MTHFR C677T SNP \[12,13\]. Collectively, this indicated that the presence of more than one prothrombotic risk factor precipitates a substantial risk of VTE \[19\]. Here, we investigate the prevalence of the prothrombotic SNPs FV-Leiden, PRT G20210A, and MTHFR C677T, in 198 patients with idiopathic DVT, compared to 697 healthy subjects.

**Subjects and Methods**

**Study group**

The demographics of study participants are summarized in Table 1. The study consisted of 198 DVT patients comprising 84 males and 114 females (age, 38.2 ± 11.4 years). DVT was diagnosed according to Doppler ultrasound (continuous wave Doppler sonography), duplex scan (color flow duplex sonography), D-Dimer levels and phlebogram. Where DVT could not be confirmed by ultrasound, D-Dimer levels (determined by ELISA) were of great diagnostic value. A normal (but not high) D-Dimer level has a high negative predictive value, and thus excluded DVT diagnosis. Phlebogram was performed as a secondary diagnostic procedure, especially in calf vein thrombosis, where the results of ultrasound were not conclusive. As control, 697 healthy individuals comprising 299 males and 398 females (age, 33.4 ± 11.8 years) were included, and were matched to patients with regards to age \((p = 0.234)\), gender \((p = 0.935)\), and residence. Exclusion criteria included personal or family history of thrombosis, cardiovascular disease, and diabetes. All subjects were Lebanese, and represent the two major sectarian groups (Moslem:Christian ratio, 64:134 and 294:403 for patients and controls, respectively). Higher percentage of smokers was found among patients (29.3 vs. 20.9%; \(p = 0.018\)). All participants were asked to sign a consent form indicating their acceptance to participate in the study, which was conducted after all institutional ethics requirements were met. EDTA-anticoagulated blood (5 ml sample) was obtained from each participant, and was processed shortly thereafter.

**Mutation analysis**

PCR-restriction fragment length polymorphism (RFLP) analysis was used for the genotype analysis. A typical PCR comprised genomic DNA (50–200 ng), Taq DNA polymerase (2.5 U; Life Technologies, Paisley, UK), 1.0 mM MgCl\(_2\), dNTP mixture (0.2 mM final concentration for each of dNTP; Promega), and 0.2 μM of sense and anti-sense oligonucleotide primers (Interactiva, Ulm, Germany), in a volume of 50 μl. PCR conditions consisted of an initial denaturation at 95°C for 3 min, followed by 36 cycles of denaturation (95°C for

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**Table 1. Demographics of Study Participants**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group</th>
<th>Patients</th>
<th>Controls</th>
<th>(P^1)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>Number</td>
<td></td>
<td>198</td>
<td>697</td>
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<td></td>
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</tr>
<tr>
<td>Gender</td>
<td>Males</td>
<td>84 (42.9)(^2)</td>
<td>299 (42.4)</td>
<td>0.935</td>
<td>1.020</td>
<td>0.741–1.403</td>
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<tr>
<td></td>
<td>Females</td>
<td>114 (57.6)</td>
<td>398 (57.1)</td>
<td></td>
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<tr>
<td>Smokers</td>
<td></td>
<td>58 (29.3)</td>
<td>146 (20.9)</td>
<td>0.018</td>
<td>1.564</td>
<td>1.099–2.236</td>
</tr>
<tr>
<td>Religion</td>
<td>Moslems</td>
<td>64 (32.3)</td>
<td>294 (42.2)</td>
<td>0.014</td>
<td>1.527</td>
<td>1.094–2.132</td>
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<td></td>
<td>Christians</td>
<td>134 (67.7)</td>
<td>403 (57.8)</td>
<td></td>
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<tr>
<td>Age groups</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>≤45 years</td>
<td>178 (89.9)</td>
<td>602 (86.4)</td>
<td>0.234</td>
<td>0.712</td>
<td>0.437–1.201</td>
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<td></td>
<td>&gt;45 years</td>
<td>20 (10.1)</td>
<td>95 (13.6)</td>
<td></td>
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<tr>
<td>Region</td>
<td>Beirut</td>
<td>82 (41.4)</td>
<td>281 (40.5)</td>
<td>0.845</td>
<td>1.047</td>
<td>0.761–1.443</td>
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<td>Mount Leh.</td>
<td>48 (24.2)</td>
<td>169 (24.4)</td>
<td>0.926</td>
<td>1.000</td>
<td>0.696–1.450</td>
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<td>South</td>
<td>25 (12.6)</td>
<td>88 (12.7)</td>
<td>0.904</td>
<td>1.000</td>
<td>0.632–1.622</td>
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<td>North</td>
<td>28 (14.1)</td>
<td>124 (17.9)</td>
<td>0.272</td>
<td>0.761</td>
<td>0.495–1.197</td>
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<td>Bekaa</td>
<td>11 (5.6)</td>
<td>27 (3.9)</td>
<td>0.403</td>
<td>1.460</td>
<td>0.737–3.033</td>
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<td></td>
<td>Others</td>
<td>4 (2.0)</td>
<td>4 (0.6)</td>
<td>0.139</td>
<td>3.572</td>
<td>0.956–13.298</td>
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