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Single Nucleotide Polymorphism in Patients with Vein Thrombosis in the Population of Latvia

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Abstract

Background: Deep vein thrombosis (DVT) is the result of innate thrombotic tendency and nongenetic triggers. Recent studies have found an association between DVT and single nucleotide polymorphisms (SNPs) in a 4q35.2 locus that contains the gene encoding factor XI (*F11*), a cytochrome P450 family member (CYP4V2).

Objective: We investigated the association of 8 SNPs genes *F5* (rs6025), *F2* (rs1799963), *SELE* (rs5361), *SERPINC1* (rs2227589), *FGG* (rs20066865), *CYP4V2* (rs13146272), *F11* (rs2289252), *GP6* (rs1613662) with DVT patients in Latvia.

Methods: The study group consisted of 99 DVT patients. The control group consisted of 99 individuals. Diagnostics was based on clinical and duplex ultrasound data (Philips Sparq). Genotyping was performed using Biosystems TaqMan SNP Genotyping Assay according to the manufacturer's protocol in ViiA[™] 7 real-time PCR system. Statistical analysis was performed using Plink 1.06. software. All genotyped polymorphisms corresponded to the Hardy-Weinberg equation and the efficacy of first time SNP genotyping was 98.8%. The additive inheritance model was used in logistic regression analysis and adjusted for gender, age and body mass index.

Results: Polymorphism of gene *F11* (rs2289252) was significantly associated with an increased risk for DVT (OR=2.19 (p=0.0004)). *F11* (rs2289252) statistically significant association with DVT remained after permutation tests and Bonferroni correction tests (p_{perm} =0.0016, after Bonferroni correction p=0.0032).

Conclusion: We found that polymorphism of gene F11 (rs2289252) was associated with an increased risk of DVT.

Keywords: Single nucleotide polymorphisms; Vein thrombosis; Deep vein thrombosis

Introduction

DVT and pulmonary embolism (PE) are major health problems with potential serious outcomes [1]. DVT is often the initial manifestation of venous disorders and is a significant health problem, causing serious consequences [1]. Epidemiological data indicate that in North America and Europe, the DVT annual incidence is 160/100,000, 20/100,000 for symptomatic non-fatal PE and 5/100,000 autopsy-detected PE [1]. Approximately one third of patients with symptomatic venous thromboembolism (VTE) manifest PE, whereas two thirds manifest DVT alone [2].

Virchow's triad plays a key role in the development of DVT [3]. Family and twin studies indicate that genetics accounts for about 60% of the risk of DVT [4,5]. Resent genetic studies of DVT have reported that several common SNPs in the 4q35.2 locus were associated with DVT [6]. These SNPs were located in three genes. Two of the genes are involved in the intrinsic blood coagulation cascade that encodes factor XI (*F11*) and prekallikrein (*KLKB1*) [6]. Two SNPs (rs2289252 and rs2036914 in *F11*) are independently associated with DVT [6]. The third gene encodes cytochrome P450, family 4, subfamily V, polypeptide 2 (CYP4V2). Multiple SNP in *GP6* (rs161662), *SERPINC1* (rs2227589), *F11* (rs2036914 and rs2289252), *FGG* (rs2066865) and *F12* (rs1801020) genes are associated with an increased risk of DVT [7].

Methods

The study group consisted of 99 patients with proven DVT. The inclusion criteria for the study group: patient is aged 18 years or above, patient is not mentally challenged, patient is diagnosed with one of the following diseases-DVT, post-thrombotic changes in deep veins (PTS), DVT for the first time, recurrent DVT, DVT with positive family history, particularly severe DVT, atypical DVT localisation, DVT with no known

reason, DVT with known reason (surgery, trauma, oncological disease), optional criterion-family history of DVT. Diagnostics was based on clinical and duplex ultrasound data (Philips Sparq). The control group consisted of 99 individuals selected from the Genome database of the Latvian population (LGDB). Individuals with diagnoses I11 to I99 (according to ICD10) were not included in the control group. The study group and control group were matched for sex proportion, average age and BMI. The control group characteristics: for DVT patients control group 35 men and 64 women, mean age 56.1 ± 15.3 years, BMI 29.3 ± 5.3 . The adjustment for sex, age, BMI was performed to exclude the possible influence of these factors on association results, as the groups were closely matched, however, not identical and it was not possible to ensure matching of the groups in stratification analysis.

Genotyping was performed using Biosystems TaqMan SNP Genotyping Assay according to the manufacturer's protocol in ViiA[™] 7 real-time PCR system. Statistical analysis was performed using Plink 1.06. software. All genotyped polymorphisms corresponded to the Hardy-Weinberg equation and the efficacy of first time SNP genotyping was 98.8%. The additive inheritance model was used in logistic regression analysis and adjusted for gender, age and body mass index.

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In the study, we used data from patients diagnosed with DVT and analysed their association with eight genes SNP.

Table I shows phenotypic characteristics of DVT group 35 (35%) men and 64 (65%) women, average age 56.2 \pm 14.7 years, BMI 28.6 \pm 5.6.

Commonly the patients of the study group were middle-aged women with increased BMI. We compared the frequency of risk alleles in patient and control groups-risk alleles did not show significant results (Table II).

Table III shows the association of analysed SNPs in the DVT group. Polymorphism of gene *F11* (rs2289252) was significantly associated with an increased risk for DVT (OR=2.19 (p=0.0004)). *F11* (rs2289252) statistically significant association with DVT remained after permutation tests and Bonferroni correction tests (p_{perm} =0.0016, after Bonferroni correction p=0.0032) (Table III). Polymorphism of

genes F5 (rs6025), F2 (rs1799963), SELE (rs5361), FGG (rs2066865), CYP4V2 (rs13146272), SERPINC1 (rs2227589) and GP6 (rs1613662) did not show a significant relationship with DVT (Table III).

Discussion and Conclusion

In this study, we explored the role of eight SNPs previously related to the DVT conditions in 99 individuals of the Latvian population. Previous studies have noted the association between DVT and the number of SNPs in the 4q35.2 locus [6]. The results of our study are similar to Li Y et al. investigated which of the 11 SNPs were independently associated with DVT by using forward stepwise logistic regression to select associated SNPs in the combined LETS, MEGA-1 and MEGA-2 studies [6]. The three SNPs selected were: rs2289252 in F11, rs2036914 in F11, and rs13146272 in CYP4V2. These same three SNPs were most strongly associated with DVT when all 11 SNPs were forced into the regression model [8]. Li et al. found that two SNPs in F11 appear to be independently associated with DVT: rs2289252 and rs2036914 [6].

Table I: Phenotypic characteristics of patients with DVT study group.

Characteristic	DVT (99)	Controls (99)	<i>p</i> -value
Male, n (%)	35 (35%)	35 (35%)	1
Female, n (%)	64 (65%)	64 (65%)	1
Mean age, (± SD) years	56.2 ± 14.7	56.1 ± 15.3	0.9626
Mean BMI, (± SD) kg/m ²	28.6 ± 5.6	29.3 ± 5.3	0.3675

Table II: Characteristics of the genotyped SNPs in patients with DVT (I80) sample group.

SNP	Conomo nosition	Minor	MAF controls	MAF cases	<i>p</i> -value		
(Gene) Ger	Genome position	allele					
rs6025 (<i>F5</i>)	140741731	т	0.01515	0.0404	0.133		
rs1799963 (<i>F2</i>)	46466972	A	0.0101	0.02525	1		
rs5361 (<i>SELE</i>)	140923391	G	0.08081	0.06566	1		
rs2227589 (SERPINC1)	145111334	т	0.1465	0.08586	0.4823		
rs2066865 (FGG)	151265852	A	0.2475	0.3182	1		
rs13146272 (<i>CYP4V2</i>)	182872291	С	0.298	0.2929	0.3927		
rs2289252 (F11)	182958773	т	0.3636	0.5455	0.8862		
rs1613662 (<i>GP6</i>)	51859927	G	0.08586	0.09091	1		
MAF-Minor allele frequency.							

Table III: Association of analysed SNPs in patients with DVT sample group.

SNP (Gene)	0 - m - th - m - r	Genotype distrib	Genotype distribution		
	Genotypes	Cases	Controls	OK (95% CI)"	<i>p</i> -value
rs6025 (F5)	TT/TC/CC	1/6/92	0/3/96	2.581 (0.6971-9.553)	0.1557
rs1799963 (F2)	AA/AG/GG	0/5/94	0/2/97	2.814 (0.5265-15.04)	0.2263
rs5361 (SELE)	GG/GT/TT	0/13/86	1/14/84	0.7814 (0.3629-1.682)	0.5285
rs2227589 (SERPINC1)	TT/TC/CC	0/17/82	1/27/71	0.5019 (0.2572-0.9795)	0.04332**
rs2066865 (FGG)	AA/AG/GG	12/39/48	4/41/54	1.385 (0.8828-2.174)	0.1563
rs13146272 (CYP4V2)	CC/CA/AA	11/36/52	9/41/49	0.9871 (0.6483-1.503)	0.9516
rs2289252 (F11)	TT/TC/CC	25/58/16	15/42/42	2.193 (1.419-3.39)	0.0004049***
rs1613662 (GP6)	GG/GA/AA	1/16/82	0/17/82	1.058 (0.5204-2.149)	0.8771

*p-value and OR were calculated using logistic regression adjusted for sex, age, BMI; **rs2227589-100,000 permutations p_{perm}=0.2347, after Bonferroni correction p=0.3466; ***rs2289252-100,000 permutations p_{nerm}=0.0016, after Bonferroni correction p=0.003239

We found a significant association of F11 rs2289252 with DVT in our sample group (Table III) and these results are similar to the results of LETS, MEGA-1, MEGA-2 studies [6-8]. The Leiden Thrombophilia Study (LETS) included individuals 18-70 years old without cancer: 443 cases with a first confirmed DVT and 453 controls with no history of DVT or pulmonary embolism (PE) [8]. The Multiple Environmental and Genetic Assessment of risk factors for the venous thrombosis (MEGA) study included individuals 18-70 years of age who presented with their first diagnosis of DVT or PE at any of six anticoagulation clinics in the Netherlands [9]. El-Galaly et al. found the association of F11 rs2289252 with venous thromboembolism, a slight tendency towards association was observed for rs1613661 in *GP6*, but not for rs1613662 [7].

In our study, we investigated polymorphisms of gene F5 (Leiden) (rs6025) in DVT patients in the Latvian population, but the result of our study is different (Table III), from the data of Bezemer et al. [10]. Our study did not show an association between polymorphisms of gene F5 (Leiden) (rs6025) and increased DVT risk. Bezemer et al. [10] described that the incidence of DVT is 1/1000 person-years [11]. The 10-year recurrence risk is 30% [12]. DVT can lead to lifethreatening PE [13]. Two more common genetic variants, Factor V Leiden and prothrombin G20210A, have been consistently found to be associated with DVT but still only explain the fraction of the DVT events [10,14-16]. Insufficient coagulation inhibitors, caused by R506Q (rs6025) mutation in the F5 gene (factor V Leiden), occurs in around 20-30% of patients with DVT, but the frequency of heterozygotes and homozygotes in the population is only 2.5-5% and 1% respectively [17]. Deficiencies of natural anticoagulants antithrombin, protein C, and protein S are strong risk factors for DVT; however, the variants causing these deficiencies are rare and explain only about 1% of all DVTs [10,14]. It has been suggested that two or more risk factors are needed for thrombosis [10,14,18,19].

According to the results of our study, polymorphism of gene F2 (rs1799963) is not associated with increased DVT risk (Table III), which differs from the results of Bezemer et al. [10]. The F2 gene (encodes coagulation factor II or prothrombin) allelic variant 20210A in 3'-non-translated region correlates with an increased level of circulating prothrombin. The F2 gene mutation frequency in the European population is 1-5%, and its occurs in 6-7% of patients with DVT [3,17].

In our study, we investigated gene SNP CYP4V2 (rs13146272), and found that rs13146272 did not show an association with increased DVT risk in our study population; the result is different (Table III) from the data of Bezemer et al. [10]. The rs13146272 SNP in the gene CYP4V2 was most strongly associated with DVT in the SNP association study [10].

In LETS, 19 682 SNPs were investigated by comparing the allele frequencies of patients and controls using a pooled DNA sample [20]. It was found that 1206 of these 19 682 SNPs were associated with DVT. These 1206 SNPs were then investigated in patients and controls from MEGA-1 using pooled DNA samples. The SNPs that were associated with DVT in both LETS and MEGA-1 were confirmed by genotyping in both studies, and it was found that 18 SNPs were consistently (with the same risk allele) associated with DVT in both LETS and MEGA-1 [10]: *MET* (rs2237712), *EPS8L2* (rs3087547), *CASP8AP2* (rs369328), *SELP* (rs6131), *ZNF544* (rs6510130), *RGS7* (rs670659), *TACR1* (rs881), *CYP4V2* (rs13146272), *F5* (rs4524), *SMOYKEEBO/F5* (rs6016), *C1orf114* (rs3820059), *F9* (rs6048), *ODZ1* (rs2266911), *F5* (Leiden) (rs6025), *F2* (G20210A). In our study, we investigated 2 genes SNP *F5* (Leiden) (rs6025), *CYP4V2* (rs13146272) from the mentioned 18 genes, the results differ (Table III) from the data of Bezemer et al. [10].

According to the results of our study, polymorphism of genes F2 (rs1799963), SERPINC1 (rs2227589), SELE (rs5361), FGG (rs20066865)

and *GP6* (rs1613662) is not associated with increased DVT risk (Table III) in our study population, which differs from the results of LETS, MEGA-1 and MEGA-2 studies [6-8], where the association of SNPs in the genes mentioned above with increased risk of DVT was demonstrated [6-8].

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Conflict of Interest

The authors report no conflict of interest.

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