RESEARCH LETTER

Methylenetetrahydrofolate reductase (*MTHFR*) gene c.665C>T and c.1286A>C and *SERPINE1* –675 4G/5G polymorphisms in Polish patients with venous thromboembolism and cryptogenic ischemic stroke

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Introduction 5,10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme involved in the remethylation of homocysteine to methionine. Two common variants of the gene *MTHFR* (1p36.22), c.665C>T and c.1286A>C, reduce the enzyme activity, increasing plasma homocysteine levels by 10% to 70%.[1] In the past, these variants have been linked to thromboembolism; however, determination of *MTHFR* polymorphisms in thrombophilia screening is currently not recommended by scientific societies.[1,2] Similarly, the single base guanine insertion / deletion polymorphism –675 4G/5G in the *SERPINE1* gene (7q22.1) that encodes plasminogen activator inhibitor type-1 (PAI-1) and has been reported to be associated with higher PAI-1 activity[3] failed to be consistently linked to venous or arterial thrombosis.[4] Despite the literature data and recommendations, commercially available thrombophilia screening panels include not only factor (F) V Leiden in the *F5* gene and the c.20210G>A prothrombin (*F2*) gene variant, but also the 3 abovementioned polymorphisms, which leads to overdiagnosis of inherited thrombophilia. Due to a paucity of data in Polish patients with venous thromboembolism (VTE) and stroke,[5-7] we assessed the frequency of the 3 gene variants in patients with a first-ever or recurrent unprovoked VTE or cryptogenic stroke.

Patients and methods Patients with a history of a first-ever and recurrent unprovoked VTE as well as those with documented cryptogenic ischemic stroke were recruited at the Center for Coagulation Disorders, Kraków, Poland for diagnostic work-up due to a suspicion of inherited thrombophilia. An unprovoked VTE episode was defined as documented pulmonary embolism or deep vein thrombosis in the absence of a history of cancer, surgery under general anesthesia, major trauma, plaster cast or hospitalization in the last month, and pregnancy or delivery in the last 3 months.[8] Recurrent VTE was defined as the occurrence of 2 or more documented unprovoked VTE episodes. Cryptogenic stroke was defined as symptomatic cerebral ischemia of obscure or unknown origin, documented on brain imaging.[8] Healthy individuals without a family history of VTE and stroke served as controls. This retrospective study was performed as part of diagnostic evaluation; thus, the approval of a bioethical committee was not required.

Inherited thrombophilia screening was performed, including FV Leiden and 20210G>A *F2* mutations, along with protein C (PC), protein S (PS), or antithrombin (AT) deficiency, as previously described.[9] The *MTHFR* c.665C>T and *MTHFR* c.1286A>C single nucleotide polymorphisms (SNPs) were genotyped using TaqMan SNP assays (Applied Biosystems, Thermo Fisher Scientific, Foster City, California, United States; assay IDs for the *MTHFR* rs1801133 and *MTHFR* rs1801131, C__1202883_20 and C__850486_20, respectively). The *SERPINE1* SNP was genotyped using a polymerase chain reaction kit (Applied Biosystems) solely in VTE patients and controls.

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TABLE 1 Distribution of polymorphic variants in the study group stratified according to the diagnosis in comparison with healthy controls

Variable	Genotype	Healthy controls (n = 184)	First-ever unprovoked VTE (n = 256)	*P* value	Recurrent unprovoked VTE (n = 166)	*P* value	Cryptogenic ischemic stroke (n = 213)	*P* value
Age, y, mean (SD)	–	46.3 (12.37)	40.9 (11.45)	<0.001	56.8 (10.36)	<0.001	45.9 (10.87)	0.99
Women, n (%)	–	108 (58.7)	124 (48.4)	0.20	92 (55.4)	0.93	130 (61.0)	0.99
MTHFR c.665C>T, n (%)	CC	87 (47.3)	120 (46.9)	0.93	89 (53.6)	0.41	94 (44.1)	0.63
	CT	82 (44.6)	106 (41.4)	0.58	56 (33.7)	0.10	96 (45.1)	0.98
	TT	15 (8.1)	30 (11.7)	0.19	21 (12.7)	0.14	23 (10.8)	0.31
MTHFR c.1286A>C, n (%)	AA	94 (51.1)	116 (45.3)	0.39	80 (48.2)	0.70	108 (50.7)	0.97
	AC	73 (39.7)	105 (41.0)	0.89	68 (41.0)	0.85	88 (41.3)	0.86
	CC	17 (9.2)	35 (13.7)	0.13	18 (10.8)	0.64	17 (8.0)	0.78
SERPINE1 –675 4G/5G, n (%)	5G5G	37 (20.1)	37 (15.4)[a]	0.24	36 (22.6)[b]	0.61	–	–
	4G5G	89 (48.4)	126 (52.3)[a]	0.57	71 (44.7)[b]	0.61	–	–
	4G4G	58 (31.5)	78 (32.4)[a]	0.88	52 (32.7)[b]	0.85	–	–

a Data available for 241 patients

b Data available for 159 patients

Abbreviations: MTHFR, methylenetetrahydrofolate reductase; VTE, venous thromboembolism

Statistical analysis Variables were presented as numbers with percentages or means with SD. Normality was assessed by the Shapiro–Wilk test. A generalized linear model was used to identify the effect of age, sex as well as *MTHFR* c.665C>T, *MTHFR* c.1286A>C, and *SERPINE1* –675 4G/5G SNPs on the probability of VTE or stroke. Comparisons between the groups and assessment of deviation from the Hardy–Weinberg equilibrium were performed by the Pearson χ^2 test. A *P* value of less than 0.05 was considered significant. Statistical analysis was performed using StatSoft Statistica 13.3 (TIBCO, Palo Alto, California, United States).

Results The main characteristics of patients are shown in TABLE 1. There were 256 individuals with unprovoked VTE, including 43 individuals (16.8%) heterozygous for FV Leiden, 13 (5.1%) heterozygous for 20210G>A *F2*, 31 (12.1%) with PC deficiency, 45 (17.6%) with PS deficiency, and 5 (2%) with AT deficiency. Recurrent VTE was diagnosed in 166 patients, including 27 individuals (16.3%) heterozygous for FV Leiden, 10 (6%) heterozygous for 20210G>A *F2*, 19 (11.5%) with PC deficiency, 23 (13.9%) with PS deficiency, and 10 (6.0%) with AT deficiency. Among the 213 patients with cryptogenic ischemic stroke, 9 (4.2%) individuals were heterozygous for FV Leiden, 8 (3.8%) were heterozygous for 20210G>A *F2*, 5 (2.6%) had PC deficiency, 11 (5.2%) had PS deficiency, and 4 (1.9%) had AT deficiency.

As shown in TABLE 1, the *MTHFR* c.665C>T minor allele frequency was 0.32 in the group of patients with a first-ever unprovoked VTE, 0.30 in those with recurrent VTE, 0.33 in patients with cryptogenic ischemic stroke, and 0.30 in controls. The *MTHFR* c.1286A>C minor allele frequency was 0.34 in patients with a first-ever unprovoked VTE, 0.31 in those with recurrent VTE, and 0.30 in patients with cryptogenic ischemic stroke. In the control group, the *MTHFR* c.1286A>C minor allele frequency was 0.29. Double mutant homozygotes or compound heterozygotes were not found. The *SERPINE1* –675 4G/5G minor allele frequency was 0.55 in the first-ever unprovoked VTE group, 0.53 in patients with recurrent VTE, and 0.56 in the control group. There were no differences between controls and the 3 study subgroups with regard to the frequency of any of the genetic variants tested (TABLE 1). After adjustment for age there were no differences in allelic distribution of the studied polymorphisms (all *P* >0.05, data not shown). There was no deviation from the Hardy–Weinberg equilibrium regarding the 3 investigated polymorphisms in the study groups (all *P* >0.05).

Discussion To the best of our knowledge, the current study is the most comprehensive to show the frequency of common *MTHFR* and *SERPINE1* polymorphisms in the Polish population, mostly from the south of Poland (the Małopolska region). We found high minor allele frequencies of the *MTHFR* c.665C>T and c.1286A>C variants and *SERPINE1* –675 4G/5G polymorphisms, which were similar in patients with unprovoked (0.32, 0.34, 0.55) and recurrent VTE (0.30, 0.31, 0.53) as compared with healthy controls (0.30, 0.29, 0.56, respectively). The minor allele frequencies of the *MTHFR* c.665C>T and c.1286A>C variants and *SERPINE1* –675 4G/5G polymorphisms are consistent with the data observed in patients with VTE and stroke from Europe (0.32, 0.34, 0.49) and the United States (0.30, 0.31, 0.54, respectively).[10-12] Our study provides new information on the frequency of the *MTHFR* and *SERPINE1* polymorphisms investigated in the largest Slavic population from Central-Eastern Europe, which constitutes a valuable confirmation of previous results.

Robust data demonstrated no relationship between either of the *MTHFR* polymorphisms and VTE in Whites.[12] In 149 Polish patients with both provoked and unprovoked VTE, recruited in the Małopolska region more than 20 years ago, no association between VTE and the *MTHFR* polymorphisms was observed, nor was it found in a subgroup analysis of 146 patients,[6] with similar minor allele frequencies in the study and control groups (*MTHFR* c.665C>T, 0.25 vs 0.25; *MTHFR*, c.1286A>C 0.19 vs 0.25, respectively). In the present study, performed in a larger population of patients with first-ever or recurrent unprovoked VTE episodes, the frequencies were slightly higher, in particular with regard to the *MTHFR* c.1286A>C variant, which is likely due to the differences in patient characteristics.

Regarding ischemic stroke, Łopaciuk et al[7] examined 100 patients aged up to 45 years following noncardioembolic ischemic stroke and reported frequencies of the *MTHFR* 665TT, CT, and CC genotypes of 12%, 37%, and 51%, respectively; the frequencies were similar to those found in controls (11%, 40%, and 49%, respectively). Unexpectedly, the *MTHFR* c.665C>T minor allele frequency was found to be higher in patients with ischemic stroke (including those with an established cause; n = 152), originating from north-western Poland as compared with the control group of 135 consecutive newborns (34.5% vs 21.5%; $P < 0.01$). The difference in the frequency of the *MTHFR* c.665TT genotype was also significant (11.8% vs 4.4%; $P < 0.01$).[13] We observed higher proportions of heterozygous patients and controls as well as homozygous controls, which is more in line with the literature data from Europe.[1,2,7] These differences are probably related to the patient selection criteria in our study and former studies.[1,2,7] To our best knowledge, the current study is the first to show the allelic frequency of the *MTHFR* c.665C>T and c.1286A>C variants in a single group of adult patients with cryptogenic ischemic stroke from Poland with no difference as compared with controls, which confirms that there is no benefit derived from determination of these variants in European populations of patients with stroke.

The *SERPINE1* –675 4G/5G minor allele frequencies detected in our study (0.53–0.56) are slightly higher than those reported in patients with VTE from Germany (0.44–0.49) and France (0.47), and similar to the values observed in the United Kingdom (0.53).[10] To our best knowledge, we are the first to show data on these genetic variants in Polish VTE patients.

The present study has some limitations. First, we did not measure homocysteine levels and PAI-1 activity. However, current data speak against the impact of the tested polymorphisms on the risk of VTE or stroke[1-4]; therefore, additional tests should not affect the conclusions. Second, the *SERPINE1* –675 4G/5G polymorphism was not assessed in stroke patients; however, most studies from Europe showed no evidence suggesting its role in this disease.[14] The effect of anticoagulant therapy and comorbidities as well as long-term prognosis in relation to the genetic variants tested were beyond the scope of the current study.

In conclusion, we found no differences between the frequency of common *MTHFR* and *SERPINE1* gene variants in patients with VTE and cryptogenic ischemic stroke compared with healthy controls. Therefore, determination of these variants should not be included in the panel of tests for congenital thrombophilia due to their high prevalence, similar to that in healthy individuals.

The presence of common *MTHFR* and *SERPINE1* mutations does not increase the risk of VTE or ischemic stroke. It should be highlighted that detection of any of the 3 genetic variants for clinical purposes may lead to misinterpretation of the results, entail unnecessary expense, and cause distress for the patients. For this reason, testing for the 3 polymorphisms should be strongly discouraged in clinical practice.

ARTICLE INFORMATION

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