PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q, and Prothrombin 20210A in Splanchnic Vein Thrombosis: Analysis of Individual Patient Data From Three Prospective Studies

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Background: There are no univocal opinions on the role of genetic thrombophilia on splanchnic vein thrombosis (SVT). We defined genetic thrombophilia the presence of one of these thrombophilic genetic factors (THRGFs): PAI-1 4G-4G, MTHFR 677TT, V Leiden 506Q, and prothrombin 20210A. **Objectives:** To evaluate the frequencies of these THRGFs in SVT patients, we analyzed individual data of 482 Caucasian patients, recruited from 2000 to 2014 in three prospective studies. SVT was defined as the presence of thrombosis of portal (PVT), mesenteric (MVT), splenic (SPVT), cava (CT), and hepatic vein (Budd Chiari syndrome, BCS). Pre-hepatic SVT (pre-HSVT) was defined as PVT with or without MVT/SPVT, without BCS. Post-hepatic SVT (post-HSVT) was BCS with or without PVT/MVT/SPVT. **Methods:** We compared 350 patients with liver cirrhosis (LC), 47 hepatocellular carcinoma (HCC), 37 myeloproliferative neoplasm (MPN), 38 associated disease (AD), 10 without any associated disease (WAD), vs 150 healthy controls (HC); 437 patients showed pre-HSVT and 45 post-HSVT. **Results:** Thrombophilia was present in 294/482 (60.9%) patients: 189/350 LC (54.0%), 31/47 (66.0%) HCC, 29/39 (74.4%) MPN, 35/38 AD (92.1%), and 10/10 (100%) WAD, and 54/150 (36.0%) in HC. In the total group, we found 175 PAI-1 4G-4G, 130 MTHFR 677TT, 42V Leiden 506Q, and 27 prothrombin 20210A; 75 patients showed presence of >1 TRHGF; the more frequent association was PAI-1 4G-4G/MTHFR 677TT, in 36 patients. PAI-1 4G-4G and MTHFR 677TT were significantly more frequent in patients with SVT (*P* values <0.005), whereas V Leiden Q506 and prothrombin G20210A were not. PAI-1 4G-4G and MTHFR 677TT distributions deviated significantly from that expected from a population in Hardy-Weinberg equilibrium. Thrombophilia was significantly less frequent in patients with pre-HSVT (250/437, 57.2%) than in patients with post-HSVT (44/45, 97.8%). **Conclusions:** Our study shows the significant prevalence of PAI-1 4G-4G and MTHFR 677TT in SVT, mainly in post-HSVT. (J CLIN EXP HEPATOL 2016;6:10–14)

There are no univocal opinions on the role of thrombophilic genetic factors (THRGFs) in splanchnic vein thrombosis (SVT); this depends in part on geographical and genetic differences among patients, in part because many studies did not analyze the same THRGFs. We published three articles on the prevalence of PAI-1, MTHFR C677, V Leiden 506Q, and prothrombin

20210A: the first study on liver cirrhosis (LC) and hepatocellular carcinoma (HCC),¹ the second, on abdominal thrombosis in patients without LC or HCC,² and the third on a large series of patients with LC.³ In these studies, PAI-1 4G-4G and MTHFR 677TT were significant risk factors of portal vein thrombosis (PVT) and Budd Chiari syndrome (BCS), in all groups of patients, whereas the role of V Leiden 506Q and prothrombin 20210A was significant, only in subgroups of patients: specifically V Leiden 506Q in BCS, and prothrombin 20210A in HCC patients. To compare the prevalence of these four THRGFs in patients with SVT, studied by our group, from 2000 to 2014, we analyzed individual patient data, from the three studies above described. We show the results below.

MATERIALS AND METHODS

Patients

We analyzed 482 Caucasian patients with SVT, included into three studies from 2000 to 2014. The first study included 107 patients with LC and 47 patients with

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Abbreviations: AD: associated disease; BCS: Budd Chirisynndrome; CT: cava thrombosis; HC: healthy controls; HCC: hepatocellular carcinoma; LC: liver cirrhosis; MPN: myeloproliferative neoplasm; MVT: mesenteric vein thrombosis; Pre-HSVT: pre-hepatic SVT (presence of PVT with or without MVT/SPVT, without BCS); Post-HSVT: post-hepatic SVT (BCS with or without other thrombosis sites); PVT: portal vein thrombosis; SPVT: splenic vein thrombosis; SVT: splanchnic vein thrombosis; THRGF: thrombophilic genetic factor; WAD: without any associated disease

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HCC, recruited from January 2000 to December 2007,¹ the second, 85 patients with non-cirrhotic SVT, recruited from January 2005 to June 2011,² and the third, 243 patients with LC recruited from June 2008 to January 2014.³ We built a file with demographic, clinical and genetic data from the individual patients, belonging to the three studies.

Table 1 shows the clinical characteristics of patients, declared in the previous original studies.¹⁻³ The diagnosis of LC was biopsy proven in patients without ascites and/or esophageal varices (75 in the first and 95 in the third study), and was based on compatible physical signs, laboratory and ultrasound findings in the other patients. For the diagnosis of HCC, we used the criteria of the Italian Association for the Study of the Liver.⁴

In the second study,² 85 patients with SVT without LC or HCC, entered into the study. Seventy-five patients showed the presence of some disease or particular clinical status: 37 myeloproliferative neoplasm (MPN) and 38 other associated disease (AD): 12 abdominal surgery, 10 oral contraception or pregnancy, 7 abdominal acute disease, and 9 chronic disease (specifically 3 Crohn's disease, 2 Bechet's syndrome, 1 Gaucher's syndrome, 1 paroxysmal nocturnal hemoglobinuria, 1 hemophagocytic syndrome, 1 nephrotic syndrome). We analyzed these 38 patients all together, as AD patients. Ten patients without associated disease (WAD) constituted the last group.

In the third study, we recruited 243 patients with SVT, of whom 14 with BCS.

All patients underwent to gastroscopy and we registered esophageal variceal size as large-medium/small/absent. Eventual previous bleeding episodes were also registered.

Local human research committee approved the study protocol of the three studies. All patients signed an

informed consent, and the study was conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Splanchnic Vein Thrombosis: Diagnosis Criteria

SVT was defined as the presence of one of these localizations: PVT, mesenteric vein thrombosis (MVT), splenic vein thrombosis (SPVT), BCS, and cava thrombosis (CT); moreover we defined pre-hepatic SVT (pre-HSVT), presence of PVT with or without MVT/SPVT and post-hepatic SVT (post-HSVT), presence of BCS, with or without other sites of thrombosis. PVT diagnosis was made, when unambiguous diagnostic evidence (endo luminal material and the absence of flow or presence of cavernous transformation), for extra hepatic PVT was detected by proper imaging techniques (Doppler ultrasound, computerized tomography, or magnetic resonance imaging). BCS was present, when we detected unambiguous evidence for hepatic venous outflow obstruction at any point between the level of the small hepatic veins and the entrance of the inferior vena cava into the right atrium, by proper imaging techniques, as defined above. Presence of MVT, SPVT, and CT was registered.

Thrombophilic Genetic Factors and Definition of Thrombophilia

We performed the genotyping of polymorphisms by polymerase chain reaction-restriction fragment length polymorphism. We analyzed the presence of each TGF mutation in heterozygous and homozygous and their association in patients with SVT and HC.

We defined genetic thrombophilia, when at least one of the following genetic factors was present: PAI-1 4G-4G, MTHFR C677T homozygous, V Leiden Q506 homozygous or heterozygous, prothrombin G20210A homozygous or

Table 1. Main Demographic and Clinical Characteristics in 482 Patients With Splanchnic Vein Thrombosis and Associated Liver Cirrhosis (LC), Hepatocellular Carcinoma (HCC), Myeloproliferative Neoplasm (MPN), Patients With (AD) and Without Associated Disease (WAD).

	LC	HCC	MPN	AD	WAD	Total
No. of patients	350	47	37	38	10	482
Age: median (range)	58 (19–83)	63 (36–83)	53 (21–83)	51 (27–79)	45 (25–72)	57 (19–83)
Male sex	232	35	14	21	6	308
HCV	220	32	3	0	0	255
HBV	53	13	0	0	0	66
Alcohol	45	5	1	0	0	51
>1 etiologic factor	–	5	–	–	–	5
Cryptogenic	83	2	0	0	0	85
Mesenteric and/or splenic vein thrombosis	53	14	15	4	1	87
Esophageal varices: large-medium/small/absent	241/69/40	17/9/21	13/10/14	11/8/19	2/3/5	267/90/125
Patients with previous bleeding episodes	194	11	7	13	2	216

heterozygous, according to literature data on phenotype, as in our previous studies.¹⁻³

Statistical Analysis

Each THRGF frequency was compared in the patients with SVT vs healthy controls, using odds ratio with 95% confidence interval (OR 95%CI) and χ^2 test.⁵ Moreover, we compared the observed frequencies for THRGF genotypes with those predicted in a population by the Hardy-Weinberg equilibrium, using interactive web-tool system calculator.⁶

RESULTS

There were no significant differences between the two groups of patients with LC, belonging to the two studies^{1,3}; consequently, we analyzed the 350 patients with LC together.

The whole group with SVT consisted of 482 subjects: 350 with LC, 47 patients HCC, 37 MPN, 38 AD, and 10 WAD. Table 1 shows the main demographic and clinical characteristics patients with SVT, with a constant prevalence of males near 65%, and a median age that progressively reduces in the various analyzed subgroups: LC 58 (19-83), HCC 63 (36-83), MPN 53 (21-83), AD 51 (27-79), WAD 45 (25-72) years.

According to the clinical characteristics, 222 patients with LC and 32 with HCC were HCV positive, 51 and 13 HBV positive, 45 and 5 alcohol abusers (80 g/die for >5 years). Among patients with MPN, three were HCV positive, and one was alcoholic. None of the patients showed homozygous for V Leiden Q506 or prothrombin G20210A.

Among 350 LC, 112 PAI-1 4G-4G, 89 MTHFR 677TT, 28V Leiden Q506, and 18 prothrombin 20210A, 56 patients with the association of more than one THRGF, so that 189 with at least one THRGF were found.

In the group of 47 HCC, 15 were PAI-1 4G-4G, 17 MTHFR 677TT, 1V Leiden Q506, and 4 prothrombin 20210A; four patients showed the association of more than one THRGF, so that 29 patients showed at least one THRGF.

In the group of 37 MPN, 18 PAI 4G-4G, 8 MTHFR 677T, 6V Leiden Q506 2 prothrombin 20210A, were found; five patients showed the associations of more than one THRGF, so that 29 patients showed at least one THRGF.

Among 38 AD patients, 23 PAI 4G-4G, 10 MTHFR 677T, 4V Leiden Q506, and one prothrombin 20210A were found, 3 patients with the association of more than 1 THRGF and 35 with at least one THRGF.

In the group of 10 WAD patients, 7 PAI 4G-4G, 6 MTHFR677T, 3V Leiden, and 2 prothrombin 20210A were found; 6 patients showed the association of more than one THRGF and a total of 10 patients with at least one THRGF.

Table 2 shows that the prevalence of PAI 4G-4G and MTHFR 677TT was significantly higher in all the groups of patients: in percentage, 25 and 31 in LC, 36 and 31 in HCC, 21 and 48 in MPN, 26 and 60 in AD, 60 and 70 in WAD. AD patients have a very high prevalence of PAI-1 4G-4G, and WAD patients of PAI-1 4G-4G and MTHFR 677TT. We evaluated the frequencies of each THRGF, in patients with SVT vs 150 HC; χ^2 P value and OR (95% CI) of PAI-1 4G-4G and MTHFR 677TT were found significantly more frequent, whereas V Leiden Q506 and prothrombin G20210A were not; PAI-1 4G-4G was the most frequent THRGF.

According to previous observations,⁷ the total number of mutations and the rate of total number of mutations/patient were calculated. All the groups showed a prevalence rate near two, but in WAD patients, the rate was much higher: 131 mutations in 10 patients and rate of 3.1; the

Table 2 Frequencies of Thrombophilic Genetic Factors (THRGFs), PAI-14G-4G, MTHFR677TT, V Leiden 506Q and Prothrombin 20210A in Patients With Liver Cirrhosis (LC) Hepatocellular Carcinoma (HCC), Myeloproliferative Neoplasm (MPN), With (AD) and Without Associated Disease (WAD), vs Healthy Controls (HC).

	LC (%)	HCC (%)	MPN (%)	AD (%)	WAD (%)	Total SVT (A) (%)	HC (B) (%)	OR (95% CI) (A) vs (B)	χ^2 test P value
Number of patients	350 (100)	47 (100)	37 (100)	38 (100)	10 (100)	482 (100)	150 (100)	–	–
PAI-1 4G-4G	111 (31)	15 (31)	18 (48)	23 (60)	7 (70)	174 (36)	26 (17.3)	2.70 (1.7–4.4)	18.6 0.000
MTHFR 677TT	88 (25)	17 (36)	8 (21)	10 (26)	6 (60)	129 (26)	20 (13.3)	2.38 (1.4–4.1)	11.5 0.001
V Leiden 506Q	29 (8)	1 (2)	6 (16)	4 (10)	3 (30)	42 (8)	7 (4.6)	2.0 (0.8–4.9)	2.6 0.12
Prothrombin 20210A	18 (5)	4 (8)	2 (5)	1 (2)	2 (20)	27 (5)	4 (2.6)	2.2 (0.7–7.4)	2.1 0.15
>1 THRGF	52 (14)	4 (8)	5 (13)	3 (7)	6 (60)	70 (14)	2 (1.3)	12.6 (3.0–75.1)	19.7 0.000
At least 1 THRGF	189 (54)	29 (61)	29 (78)	35 (92)	10 (100)	292 (60)	54 (36.0)	2.7 (1.8–4.1)	27.9 0.000

Table 3 Frequencies of Thrombophilic Genetic Factors (THRGFs), PAI-1 4G-4G, MTHFR 677TT, Leiden 506Q, Prothrombin 20210A, Presence of >1 THRGF and At Least 1 THRGF, in 482 Patients With Splanchnic Vein Thrombosis: 418 With Pre-Hepatic Splanchnic Vein Thrombosis (pre-HSVT): Portal With Mesenteric/Splenic Thrombosis, and 45 With Post-HSVT: Budd Chiari Syndrome With Portal/Mesenteric/Splenic/Cava Thrombosis.

	Number of patients (%)	PAI-1 4G-4G (%)	MTHFR 677TT (%)	Leiden 506Q (%)	Prothrombin 20210A (%)	>1 THRGF (%)	At least 1 THRGF (%)
Total number of patients	482 (100)	174 (36.1)	129 (26.8)	42 (8.7)	27 (5.6)	70 (14.5)	292 (60.6)
Pre-HSVT	437 (100)	**147 (33.6)	121 (27.1)	*29 (6.6)	26 (5.9)	65 (14.9)	§248 (56.7)
Post-HSVT	45 (100)	**27 (60.0)	8 (17.8)	*13 (31.0)	1 (2.2)	5 (11.1)	§44 (97.8)

* χ^2 test 37.2, $P < 0.001$.

** χ^2 test 12.3, $P < 0.001$.

§ χ^2 test 28.8, $P < 0.0001$.

other were 103 in HCC (rate 2.2), 633 in LC (rate 1.8), 89 in MPN (rate 2.3), 97 in AD (rate 2.6), and 183 (rate 1.2) in HC.

Four hundred and thirty seven patients showed pre-HSVT, and 45 patients showed post-HSVT; 101 showed more than one site of thrombosis, the more frequent PVT and MVT (83 patients).

Hardy-Weinberg (HW) equilibrium for PAI-1 and MTHFR 677, deviated from that expected from a population in equilibrium (PAI-1 χ^2 41.96, $P < 0.0001$; MTHFR 677 χ^2 14.93, P 0.0001), whereas HW equilibrium was respected (all P values >0.05) on V Leiden Q506 and prothrombin 20210A genotype distributions.

Table 3 shows the frequencies of PAI-1 4G-4G, MTHFR 677TT, Leiden 506Q, prothrombin 20210A, presence of more than 1, and at least 1 THRGF, in relation to pre- or post-HSVT. Four hundred and eighteen patients showed pre-HSVT: 354 isolated PVT and 83 PVT with MVT/SPVT; 45 post-HSVT: 26 isolated BCS, and 19 BCS with other sites of thrombosis. PAI-1 4G-4G, V Leiden 506Q, and presence of at least one THRGF, were significantly more frequent in post- vs pre-HSVT: respectively 147 (33.6%) and 27 (60.0%), $P < 0.001$; 13 (31.0%) and 29 (6.6%), $P < 0.001$; 44 (97.8%) and 248 (56.7%), P value < 0.0001 .

DISCUSSION

Our data represent, to our knowledge, the study with the largest number of patients in SVT, to date published.

The first result is the younger mean age in WAD patients: 45 vs 57 years in the total group. The second is that post-HSVT represents about 10% of SVT: 45/482 patients showed BCS: 26 isolated and 19 associated with other SVT localization. We think this is the true prevalence of post-HSVT on the totality of the SVT, observed in our geographical area of Caucasian population, because our three studies have enrolled only consecutive patients.

Another result is the high frequency of thrombophilia, about 61% (294/482 patients) with the following percentages: PAI-1 4G-4G 36, MTHFR 677TT 26, V Leiden 506Q

8, prothrombin 20210A 5, >1 THRGF 14. The percentages of the THRGFs, in relation to the disease associated, show data growing from 54 in LC, 61 HCC, 78 MPN, 92 AD, to 100 in WAD patients. This could mean that diseases, such as LC, HCC, MPN, or the acute or chronic ones, have an intrinsic tendency to develop SVT in a more or less frequent, whereas the genetic thrombophilia is practically obligatory in AD and WAD patients.

Multiple factors, namely the presence of some disease and/or thrombophilia are present in about one third of patients with BCS and two thirds of patients with PVT, in according with De Stefano et al.⁸ In the same mode, multiple factors are present in two thirds of patients with pre-HSVT, 248/437 patients (56.7%), whereas the association is nearly 100% (44 patients) among 45 patients with post-HSVT: 43 patients showed associated disease (14 LC, 17 MPN, and 12 AD), and 44 presence of thrombophilia. Frequencies of THRGFs in 437 patients with pre-HSVT and 45 with post-HSVT show that PAI-1 4G-4G, Leiden 506Q and the presence of at least 1 THRGF, were significantly more frequent, in post- vs pre-HSVT, whereas prothrombin 20210A, MTHFR 677TT, and presence of more than 1 THRGF are similar in the two groups, as shown in Table 3.

The most relevant result of the present study is the role of PAI-1 4G-4G and MTHFR 677TT in the total group of patients with SVT, as shown in Table 2, whereas the role of Factor V Leiden Q506 and prothrombin 20210A mutation is less important in this series of patients, confirming previous study.⁹ PAI-1 4G-4G has come out as the leader factor in the SVT group, and in the various subgroups, confirming its important role in pathogenesis of SVT, as Balta et al.¹⁰ have found, and confirming itself as a thrombotic risk factor in different target organs, as showed by Tsantes et al.¹¹ MTHFR 677TT genotype was strongly associated with portal thrombosis in our cirrhotic patients as reported previously by Amitrano et al.¹²

Hardy-Weinberg equilibrium for PAI-1 and MTHFR 677 was deviated from that expected from a population (PAI-1 χ^2 41.96, $P < 0.0001$; MTHFR 677 χ^2 15.00, P 0.0001).

We think that PAI-1 4G-4G and MTHFR 677TT can increase the inflammation response, participating to the activation of perisinusoidal hepatic stellate cells, causing hepatic fibrosis and augmented intrahepatic vascular resistance typical of the LC, as suggested by Fernandez.¹³

V Leiden 506Q was significantly correlated with BCS patients (13/45 patients), confirming other previous articles on BCS,^{14,15} but Hardy-Weinberg equilibrium for V Leiden 506Q was respected both in patients with SVT and in HC.

We failed to demonstrate a role of prothrombin 20210A with SVT, and it was in Hardy-Weinberg equilibrium.

What is the real reason why patients develop pre-, instead of post-HSVT remains undefined; surely, the reason should be a set of causes that present themselves together more rarely in post-HSVT, or in the different hemodynamic conditions between the pre- and post-SVT districts.

However, to assess these results, larger longitudinal studies, hopefully from diverse geographical areas, are widely needed to confirm the interrelations of all pathogenic factors involved in the complex mechanism of SVT.

In conclusion, we remark that genetic thrombophilia, present in more than 60% of patients with SVT, with a large variation of frequency, ranging from 50% in LC to 100% in WAD patients, is mainly due to PAI-1 4G-4G and/or MTHFR 677TT. We suppose that these allele polymorphisms, may increase the risk of thrombosis in patients with thrombophilic diseases, as LC, HCC, MPN, or acute and chronic ones, or in abdominal surgery, and they are almost necessary in patients without any diseases.

CONFLICTS OF INTEREST

I declare that none of the authors has actual or potential conflict of interest in relation to this article.

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