The *JAK2* 46/1 haplotype in Budd-Chiari syndrome and portal vein thrombosis

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The germline *JAK2* 46/1 haplotype has been associated with the development of *JAK2*^{V617F}-positive as well as *JAK2*^{V617F}-negative myeloproliferative neoplasms (MPNs). In this study we examined the role of the 46/1 haplotype in the etiology and clinical presentation of patients with splanchnic vein thrombosis (SVT), in which MPNs are the most prominent underlying etiological factor. The single-nucleotide polymorphism rs12343867, which tags 46/1, was genotyped in 199 SVT patients. The 46/1 haplotype was overrepresented in *JAK2*^{V617F}-positive SVT patients compared with controls ($P < .01$). Prevalence of the 46/1 haplotype in *JAK2*^{V617F}-negative SVT patients did not differ from prevalence in the controls. However, *JAK2*^{V617F}-negative SVT patients with a proven MPN also exhibited an increased frequency of the 46/1 haplotype ($P = .06$). Interestingly, 46/1 was associated with increased erythropoiesis in *JAK2*^{V617F}-negative SVT patients. We conclude that the 46/1 haplotype is associated with the development of *JAK2*^{V617F}-positive SVT. In addition, our findings in *JAK2*^{V617F}-negative SVT patients indicate an important role for the 46/1 haplotype in the etiology and diagnosis of SVT-related MPNs, independent of *JAK2*^{V617F}, that requires further exploration. (*Blood*. 2011;117(15):3968-3973)

Introduction

The entity splanchnic vein thrombosis is used to indicate both the Budd-Chiari syndrome (BCS) and portal vein thrombosis (PVT). BCS is a rare disorder characterized by obstruction of the hepatic veins and/or the suprahepatic inferior vena cava.[1,2] BCS is considered primary when resulting from thrombosis and secondary when obstruction of the venous tract results from compression or invasion by a tumor. Nonmalignant, noncirrhotic PVT is another infrequent thrombotic disorder involving the splanchnic vasculature.[3] In both disorders, pathogenesis is largely dependent on the presence of systemic prothrombotic conditions that promote thrombus formation in the respective hepatic vessels.

Myeloproliferative neoplasms (MPNs) are the leading cause of SVT and are diagnosed in one-third to one-half of the patients with SVT.[4] The most common gain-of-function mutation leading to development of MPNs is the *JAK2*^{V617F} mutation, which is present in more than 95% of cases of polycythemia vera and in 50% to 60% of essential thrombocythemia and primary myelofibrosis.[5] The high prevalence of the *JAK2*^{V617F} mutation in SVT patients, approximately 35% in unselected cases, confirms the unique relationship between SVT and MPNs.[6-10] Interestingly, the *JAK2*^{V617F} mutation has proved to be an important diagnostic tool for detecting MPNs in SVT patients, considering that MPNs can be notoriously difficult to diagnose in patients presenting with SVT. Portal hypertension, resulting from pre- or postsinusoidal venous congestion, leads to hypersplenism and hemodilution—both conditions that mask the characteristic peripheral blood cell changes in MPNs. Screening for the *JAK2*^{V617F} is an objective tool for diagnosing MPNs in these patients and is now part of the standard diagnostic work-up in SVT.[8,11]

Recently, an association between a specific *JAK2* haplotype and the risk of developing *JAK2*^{V617F}-positive MPNs was demonstrated.[12-14] These studies show that the acquired *JAK2*^{V617F} mutation is preferentially found within this particular, inherited haplotype, which we refer to as 46/1. The association is strong, with the odds of developing MPNs being 3- to 4-fold higher in patients carrying 46/1 compared with noncarriers. One of the aforementioned studies also found an association between 46/1 and *JAK2*^{V617F}-negative MPNs,[12] which has since been confirmed by other studies.[15-17] Subsequently, it has been shown that 46/1 was also overrepresented in *JAK2*^{V617F}-negative MPNs carrying mutations across *JAK2* exon 12 or the *MPL* gene.[18,19] These findings are of particular interest in SVT, because 46/1 may thus represent a new molecular marker for diagnosing MPNs in *JAK2*^{V617F}-negative SVT patients.

The aim of our study was to assess whether 46/1 is associated with SVT, and to determine whether *JAK2* 46/1 is associated with distinct clinical and laboratory characteristics of SVT. To this end, we genotyped patients and healthy controls from a large European series of newly diagnosed, consecutive BCS and PVT patients.

Methods

Study design

We performed a case-control study in which patients and controls were recruited from the European Network for Vascular Disease of the Liver

Submitted November 25, 2010; accepted February 1, 2011. Prepublished online as *Blood* First Edition paper, March 1, 2011; DOI: 10.1182/blood-2010-11-319087.

The online version of this article contains a data supplement.

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(EN-Vie) study cohort, which has been described previously.[6,9] The EN-Vie cohort consists of newly diagnosed patients with BCS or PVT, consecutively enrolled and prospectively followed in 9 different European countries. At time of diagnosis and during follow-up, data concerning clinical condition and etiology and results of radiology, pathology, and laboratory assessments were collected. From October 2003 to October 2005, a total of 163 BCS patients and 138 PVT patients were enrolled in the study. In addition, 105 healthy, unrelated, population-based controls, usually neighbors or friends of the patients, fulfilling the same age criteria and without a history of thrombosis were recruited. The EN-Vie study was approved by national and, if necessary, local ethical committees. Patients and controls agreed to participate in the study by written informed consent, in accordance with the Declaration of Helsinki.

Definitions

BCS was defined as hepatic outflow obstruction regardless of the cause or level of obstruction, from the small hepatic veins to the entrance of the inferior vena cava into the right atrium. BCS was confirmed by radiographic imaging (ultrasonography, computed tomography, magnetic resonance imaging, or venography). Sinusoidal obstruction syndrome was excluded from this definition, as well as outflow obstruction occurring in the setting of heart failure, orthotopic liver transplantation, or hepatobiliary cancer. Diagnostic criteria for PVT included radiographic imaging evidence of solid material in the portal vein lumen or in its left or right branch. PVT patients with cirrhosis or abdominal malignancies as well as patients with clinical, laboratory, or imaging evidence of noncirrhotic liver disease, within a context of chronic alcoholism, viral hepatitis, autoimmune disease, Wilson disease, or iron overload were excluded.

Blood sampling

Blood samples were collected from patients at time of diagnosis and controls by venapuncture in tubes containing 0.11M trisodium citrate. DNA was extracted from whole blood according to local standard methods. DNA samples were transported to the Erasmus University Medical Center in Rotterdam and stored at −70°C until analysis.

JAK2 46/1 genotyping and *JAK2*^{V617F} mutation analysis

Granulocyte DNA samples were genotyped using a Taqman single nucleotide polymorphism (SNP) assay for rs12343867 (C/T) (Applied Biosystems). The 46/1 haplotype is tagged by the C allele of this SNP.[14] *JAK2*^{V617F} mutation analysis was performed as previously described.[8]

Statistical analysis

Results are expressed as proportions for categorical variables and as medians and interquartile range (IQR; 25th-75th percentile) for continuous variables. Comparison between categorical variables was performed with χ^2 testing and between continuous variables with the Kruskal-Wallis test. Odds ratios (ORs) for SVT associated with 46/1 and corresponding 95% confidence intervals (CIs) were calculated using logistic regression, adjusted for age and sex. To assess the association between 46/1 and SVT in the presence of other risk factors for SVT, a multivariate model was constructed in which the factor V Leiden mutation and the prothrombin G20210A variant were added, adjusted for age and sex. *JAK2*^{V617F} was present only in SVT patients and not in controls, and could therefore not be included in the multivariate model. *P* values were two-tailed and statistical significance was set at $P < .05$. All statistical analyses were conducted with PASW Statistics, Version 17.0 (SPSS).

Results

Patient characteristics

The total EN-Vie cohort contains 163 BCS and 138 PVT patients, and DNA samples for this study were available for 116 BCS patients, 96 PVT patients, and 105 healthy controls. Of these, 107 BCS patients (92%), 92 PVT patients (96%), and 100 healthy controls (95%) were successfully genotyped and included in the current analysis.

Table 1. Clinical characteristics and prothrombotic factors in patients with Budd-Chiari syndrome and portal vein thrombosis

	BCS, n = 107	PVT, n = 92
Median age, y (Q1-Q3)	38.1 (28-51)	49.8 (42-57)
Males	45 (42)	43 (47)
Inherited thrombophilia*	23 (21)	22 (24)
Protein C deficiency	2 (2)	2 (2)
Protein S deficiency	1 (1)	5 (6)
Antithrombin deficiency	3 (3)	3 (4)
Factor V Leiden mutation	12 (11)	3 (3)
Prothrombin gene G20210A	5 (5)	12 (13)
Acquired thrombophilia*	83 (78)	56 (61)
Myeloproliferative neoplasms	42 (39)	24 (26)
Polycythemia vera	24 (57)	3 (13)
Essential thrombocytosis	7 (17)	10 (42)
Primary myelofibrosis	2 (5)	4 (17)
Unclassifiable	9 (21)	7 (29)
JAK2^{V617F}-positive	34 (32)	20 (22)
History of myeloproliferative neoplasms	10 (24)	6 (25)
Antiphospholipid antibodies	27 (25)	25 (28)
Paroxysmal nocturnal hemoglobinuria	12 (21)	0 (0)
Hormonal	23 (37)	18 (37)
Systemic disorder†	12 (11)	3 (3)
Local risk factor‡	15 (14)	25 (28)
Single risk factor§	49 (46)	29 (32)
Multiple risk factors§	44 (41)	43 (47)
No risk factor	14 (9)	20 (22)

Values are n (%) unless otherwise specified.

BCS indicates Budd-Chiari syndrome; and PVT, portal vein thrombosis.

*Patients can have more than one thrombophilic factor simultaneously. Not all investigations could be performed in the individual patients.

†Behçet disease, sarcoidosis, vasculitis, or connective tissue disease.

‡Intra-abdominal inflammation, infection, or abscess.

§Single risk factor: presence of an inherited or acquired thrombophilic factor or the presence of a local risk factor. Multiple risk factors: presence of 2 or more of these risk factors.

Patient characteristics and underlying thrombophilic risk factors are shown in Table 1. In BCS patients, median age at diagnosis was 38.1 years (IQR: 28-51 years) and 45 were male (42%). Twenty-three BCS patients (21%) had an underlying inherited thrombophilic factor, and in 83 patients (78%) an acquired prothrombotic disorder was diagnosed. MPNs were present in 42 patients, of whom 34 were *JAK2*^{V617F}-positive. In PVT, median age at diagnosis was 49.8 years (IQR: 42-57 years), and 43 were male (47%). Twenty-two PVT patients (24%) were diagnosed with an inherited thrombophilia, whereas 56 patients (61%) had an acquired prothrombotic factor. MPNs were present in 24 PVT patients, of whom 20 were *JAK2*^{V617F}-positive. Median age in the controls was 36.8 years (IQR: 27-50 years), and 40 were male (40%). None of the controls carried the *JAK2*^{V617F} mutation, whereas the factor V Leiden mutation and prothrombin G20210A variant were present in 4% and 3% of the controls, respectively.

JAK2 46/1 haplotype and risk of SVT-related MPN

SNP rs12343867 genotype distributions and the OR for SVT are presented in Table 2. Genotype distribution of our control group was in Hardy-Weinberg equilibrium and similar to those reported by previous studies and the Wellcome Trust Case-Control Consortium (WTCCC).[12,17,20] In the overall group of SVT patients, there was no significant difference in frequency of the minor C allele

Table 2. Association between the *JAK2* 46/1 haplotype and patients with Budd-Chiari syndrome and portal vein thrombosis

		rs12343867 genotype			C allele		Odds ratio (95% CI)†		Odds ratio (95% CI)‡	
	N	CC	CT	TT	frequency	P*	CT vs TT	CC vs TT	CT vs TT	CC vs TT
Controls	100	7 (7)	40 (40)	53 (53)	0.27					
Splanchnic vein thrombosis										
Overall	199	23 (12)	83 (42)	93 (47)	0.32	.18	1.2 (0.7-2.0)	2.0 (0.8-4.9)	1.4 (0.8-2.6)	2.1 (0.7-5.9)
JAK2^{V617F}-positive	54 (27)	9 (17)	28 (52)	17 (31)	0.43	< .01	2.1 (1.01-4.5)	4.1 (1.3-13.2)	2.7 (1.2-6.2)	4.7 (1.3-16.7)
JAK2^{V617F}-negative, MPNs present	12 (6)	4 (33)	3 (25)	5 (42)	0.46	.06	0.6 (0.1-2.9)	5.3 (1.1-26.2)	0.8 (0.2-4.0)	5.1 (0.9-28.5)
JAK2^{V617F}-negative, MPNs absent	133 (67)	10 (8)	52 (39)	71 (5)	0.27	.98	1.0 (0.6-1.7)	1.1 (0.4-3.2)	1.2 (0.6-2.2)	1.1 (0.3-3.6)
Budd-Chiari syndrome										
Overall	107	16 (15)	46 (43)	45 (42)	0.36	.04	1.4 (0.8-2.4)	2.7 (1.01-7.1)	1.6 (0.8-3.1)	2.7 (0.9-8.2)
JAK2^{V617F}-positive	34 (32)	7 (21)	16 (47)	11 (32)	0.44	.01	1.9 (0.8-4.5)	4.8 (1.4-16.6)	2.0 (0.7-5.4)	5.3 (1.4-20.6)
JAK2^{V617F}-negative	73 (68)	9 (12)	30 (41)	34 (47)	0.33	.24	1.2 (0.6-2.2)	1.9 (0.7-5.8)	1.4 (0.7-2.9)	1.8 (0.5-6.2)
Portal vein thrombosis										
Overall	92	7 (8)	37 (40)	48 (52)	0.28	.88	1.0 (0.5-1.8)	1.3 (0.4-4.4)	1.1 (0.5-2.3)	1.4 (0.4-5.3)
JAK2^{V617F}-positive	20 (22)	2 (10)	12 (60)	6 (30)	0.40	.10	2.6 (0.9-7.6)	3.1 (0.5-20.2)	4.0 (1.2-13.7)	3.8 (0.5-27.6)
JAK2^{V617F}-negative	72 (78)	5 (7)	25 (35)	42 (58)	0.24	.57	0.7 (0.3-1.4)	0.9 (0.2-3.6)	0.7 (0.3-1.6)	0.9 (0.2-4.2)

Values are n (%) unless otherwise specified.
MPNs indicates myeloproliferative neoplasms.
**P* for C-allele frequency comparisons.
†Adjusted for age and sex.
‡Adjusted for Factor V Leiden mutation, prothrombin G2021A variant, age, and sex.

compared with the controls (32% vs 27%; $P = .18$). However, when stratified for presence of the *JAK2*^{V617F} mutation, we observed a significant difference in C allele frequency (43% vs 27%; $P < .01$) in *JAK2*^{V617F}-positive individuals compared with controls. An allele-dependent increase in risk of *JAK2*^{V617F}-positive SVT was seen in subjects with the CT genotype (OR 2.1; 95% CI = 1.01-4.5) and the CC genotype (OR 4.1; 95% CI = 1.3-13.2). In *JAK2*^{V617F}-negative SVT patients in whom MPNs were excluded, C allele frequency was comparable with that in controls (27% vs 27%; $P = .98$). However, *JAK2*^{V617F}-negative SVT patients with a proven MPN (n = 12) also showed an increased frequency of the C allele (46% vs 27%; $P = .06$). The increased risk of *JAK2*^{V617F}-negative MPNs corresponded with an OR of 5.3 (95% CI = 1.1-26.2) for subjects with the CC genotype. In a multivariate model, where the factor V Leiden mutation and the prothrombin G20210A variant were added, only minimal effect on the association between 46/1 and SVT was seen. *JAK2*^{V617F}-negative SVT patients homozygous for 46/1 had a 4- to 5-fold increased risk of an underlying MPN compared with heterozygous or noncarriers (Figure 1).

In the group of only BCS patients, the minor C allele was present more frequently than in controls (36% vs 27%; $P = .04$). The risk for BCS in subjects with the CC genotype was elevated compared with subjects with the common TT genotype (OR 2.7; 95% CI = 1.01-7.1). When we stratified for presence of the *JAK2*^{V617F} mutation, we observed a significantly higher frequency of the C allele in *JAK2*^{V617F}-positive individuals with BCS (44% vs 27%; $P = .01$), with an OR for BCS in subjects with the CC genotype of 4.8 (95% CI = 1.4-16.6). No difference in C allele frequency was observed in *JAK2*^{V617F}-negative individuals with BCS compared with controls (33% vs 27%; $P = .24$).

In the group of only PVT patients, frequency of the minor C allele was similar to that in the controls (28% vs 27%; $P = .88$). In *JAK2*^{V617F}-positive individuals with PVT, we also observed a higher frequency of the C allele than in controls (40% vs 27%; $P = .10$). The increased risk of *JAK2*^{V617F}-positive PVT in

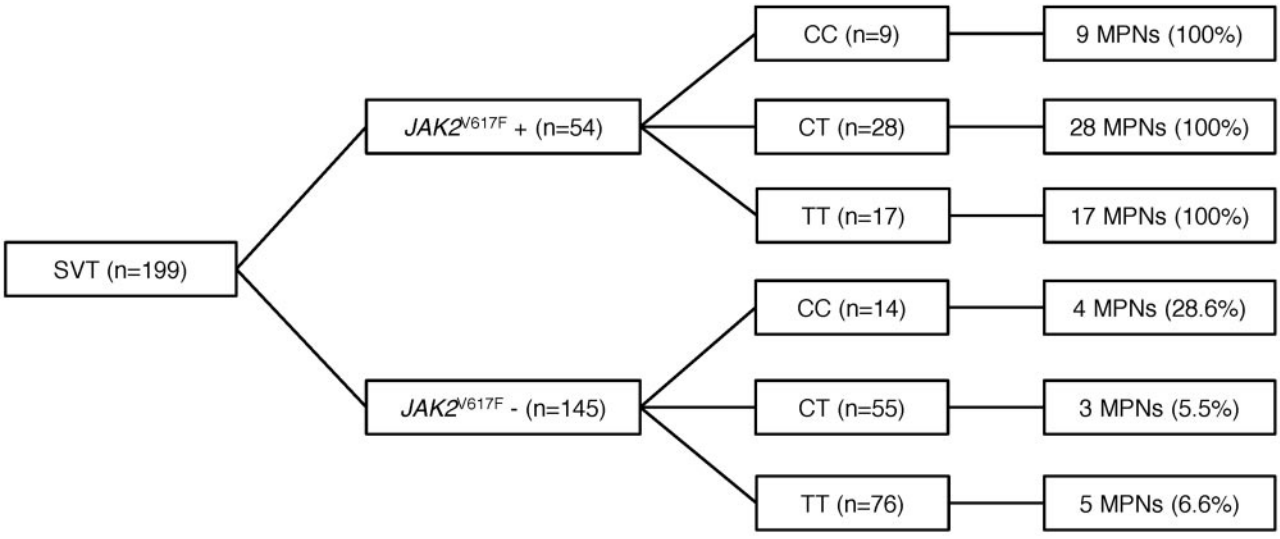

Figure 1. Number (percentage) of splanchnic vein thrombosis patients who were diagnosed with MPNs, according to rs12343867 genotype, after stratification for *JAK2*^{V617F} status. SVT indicates splanchnic vein thrombosis; MPNs, myeloproliferative neoplasms.

Table 3. Characteristics and prognostic scores associated with the *JAK2*^{V617F} mutation in 199 patients with splanchnic vein thrombosis

	JAK2^{V617F}-positive, n = 54	*JAK2*^{V617F}-negative, n = 145	*P*
Age, y	47.3 (31-54)	43.4 (31-55)	.11
Males, n (%)	20 (37)	68 (47)	.34
Hemoglobin, mmol/L	9.2 (8.2-10.4)	8.1 (6.9-9.3)	< .001
Hematocrit, %	44.5 (39-50)	39.7 (35-44)	< .001
RBC count, $\times 10^9$/L	5.2 (4.4-6.3)	4.5 (4.1-5.1)	< .01
WBC count, $\times 10^9$/L	12.5 (9.7-17.3)	8.7 (6.4-11.4)	< .001
Platelet count, $\times 10^9$/L	373 (220-538)	219 (139-347)	< .001
ALT, ULN	1.7 (0.9-5.8)	1.0 (0.7-2.2)	< .01
Serum bilirubin, μmol/L	27 (15-43)	21 (13-35)	.02
Albumin, g/L	34 (30-40)	33 (28-39)	.33
Splenomegaly, %	36 (68)	60 (42)	< .01

Continuous data are presented as median (IQR); categorical data as mean (%).
SVT indicates splanchnic vein thrombosis; RBC, red blood cell; WBC, white blood cell; ALT, alanine aminotransferase; and ULN, upper limit of normal value.

subjects with the CC genotype was 3.1 (95% CI = 0.5-20.2), which is comparable with risk in BCS patients. Also in PVT, there was no significant difference in C allele frequency in *JAK2*^{V617F}-negative individuals compared with controls (24% vs 27%; $P = .57$).

The frequency of inherited and acquired thrombophilia did not differ according to 46/1 haplotype ($P = .23$ and $P = .47$, respectively), nor was there a difference in the frequency of inherited thrombophilia between *JAK2*^{V617F}-negative and *JAK2*^{V617F}-positive SVT patients ($P = .98$). *JAK2*^{V617F} allele burden was determined in 47 of 55 SVT patients. Allele burden was determined in 47 of 55 *JAK2*^{V617F}-positive SVT patients. Frequency of the C allele was significantly increased compared with controls in SVT patients with an allele burden < 20% (30 of 74 alleles; $P = .03$), and in patients with an allele burden > 20% (11 of 16 alleles; $P < .001$).

JAK2 46/1 haplotype and relationship with clinical characteristics in SVT

First, we stratified for *JAK2*^{V617F} status and observed clear differences in hematologic and clinical features (Table 3). Hemoglobin, hematocrit, red blood cell count, white blood cell count, platelet count, serum alanine transaminase, serum bilirubin, and prevalence of splenomegaly at diagnosis were all significantly higher compared with unmutated SVT patients. Rotterdam BCS (1.18 vs 1.15; $P = .06$) and Child-Pugh (8 vs 7; $P = .05$) prognostic scores, which can be assessed only in BCS patients, were higher in *JAK2*^{V617F}-positive compared with *JAK2*^{V617F}-negative patients.

We subsequently examined the association between *JAK2* 46/1 and clinical features of SVT patients (Table 4). Higher levels of hemoglobin ($P < .01$), hematocrit ($P < .01$), red blood cell count ($P < .01$), platelet count ($P = .06$), serum alanine transaminase ($P = .04$), and a higher prevalence of splenomegaly at diagnosis ($P = .045$) were seen in SVT patients with the CC genotype compared with patients with the common TT genotype. Rotterdam BCS and Child-Pugh prognostic scores did not differ significantly according to rs12343867 genotype in BCS patients ($P = .63$ and $P = .74$, respectively).

Finally, to avoid potential confounding, we assessed the association between the rs12343867 genotype and laboratory and clinical characteristics in the *JAK2*^{V617F}-negative group (Table 4). In this analysis, *JAK2*^{V617F}-negative patients with the CC genotype had higher hemoglobin levels ($P < .01$), hematocrit ($P < .01$), and red blood cell count ($P = .02$) compared with individuals with the TT genotype (Figure 2). These associations remained significant when we excluded the 12 *JAK2*^{V617F}-negative patients in whom MPNs were objectively confirmed (data not shown). In *JAK2*^{V617F}-positive patients, we observed a higher prevalence of splenomegaly in those who were homozygous for *JAK2* 46/1 ($P = .01$), but we did not observe an association with peripheral blood cell counts (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

Discussion

Using a large multinational cohort of 199 newly diagnosed patients with SVT, we were able to show that 46/1 is associated with *JAK2*^{V617F}-positive SVT. This study also suggests an association between 46/1 and SVT in *JAK2*^{V617F}-negative SVT patients with MPNs, whereas no association was observed in *JAK2*^{V617F}-negative SVT patients without MPNs. Finally, *JAK2* 46/1 was associated with increased erythropoiesis in *JAK2*^{V617F}-negative SVT patients, which is a novel finding.

In the current analysis, the first including a large group of BCS patients, we observed an increased frequency of 46/1 in both *JAK2*^{V617F}-positive BCS and PVT. Presence of 46/1 increased the risk of occurrence of *JAK2*^{V617F}-positive BCS or PVT in an allele-dependent manner. These results are in line

Table 4. Characteristics and prognostic scores associated with the *JAK2* 46/1 haplotype in 199 patients with splanchnic vein thrombosis

	rs12343867 genotype (SVT)						rs12343867 genotype (*JAK2*^{V617F}-negative SVT)					
	CC, n = 23	CT, n = 83	TT, n = 93	*P1**	*P2**	*P3**	CC, n = 14	CT, n = 55	TT, n = 76	*P1**	*P2**	*P3**
Age, y	38.7 (31-61)	42.9 (31-52)	46.3 (34-57)	.61	.74	.33	37.9 (30-60)	42.8 (31-52)	46.0 (33-57)	.63	.76	.34
Males, %	10 (43)	31 (37)	47 (51)	.21	.54	.09	10 (71)	18 (33)	40 (53)	.01	.19	.15
Hemoglobin, mmol/L	9.5 (8.1-10.5)	8.2 (7.1-9.1)	8.3 (6.9-9.5)	< .01	< .01	.79	9.6 (7.8-10.6)	7.9 (7.0-8.8)	8.1 (6.6-9.1)	.02	< .01	.38
Hematocrit, %	47.2 (41-51)	40.0 (36-44)	40.2 (34-45)	< .01	< .01	.15	47.0 (38-51)	39.5 (36-43)	38.9 (33-43)	.02	< .01	.10
RBC count, $\times 10^9$/L	5.6 (4.8-6.3)	4.6 (4.2-5.3)	4.6 (3.8-5.2)	< .01	< .01	.24	5.4 (4.8-5.7)	4.5 (4.2-5.0)	4.4 (3.8-5.0)	.04	.02	.18
WBC count, $\times 10^9$/L	10.5 (7.3-13.7)	10.1 (7.2-13.3)	9.3 (6.5-12.5)	.34	.17	.20	8.0 (7.0-13.2)	8.8 (6.0-11.5)	8.6 (6.1-11.4)	.85	.65	.59
Platelet count, $\times 10^9$/L	357 (217-461)	235 (155-418)	231 (138-394)	.17	.06	.35	242 (167-390)	188 (141-336)	220 (124-339)	.76	.51	.85
ALT, ULN	1.3 (1.0-6.7)	1.2 (0.8-2.7)	1.0 (0.7-2.4)	.11	.04	.11	1.1 (0.9-3.6)	1.0 (0.7-2.1)	1.0 (0.6-2.3)	.58	.35	.49
Serum bilirubin, μmol/L	28 (15-44)	20 (12-36)	24 (13-38)	.32	.39	.65	22 (14-45)	17 (13-29)	22 (13-38)	.59	.79	.52
Albumin, g/L	34 (30-38)	34 (29-39)	33 (28-40)	.95	.88	.76	34 (25-35)	34 (29-39)	33 (27-40)	.83	.76	.82
Splenomegaly, %	16 (70)	38 (46)	42 (46)	.11	.045	.46	7 (100)	20 (36)	33 (45)	.53	.71	.51

Continuous data are presented as median (IQR range); categorical data as mean (%).
SVT indicates splanchnic vein thrombosis; RBC, red blood cell; WBC, white blood cell; ALT, alanine aminotransferase; and ULN, upper limit of normal value.
*P1, *P* value for genotype comparisons (CC/CT/TT); *P2*, *P* value for CC vs TT genotype comparisons; *P3*, *P* value for CC/CT vs TT genotype comparisons.

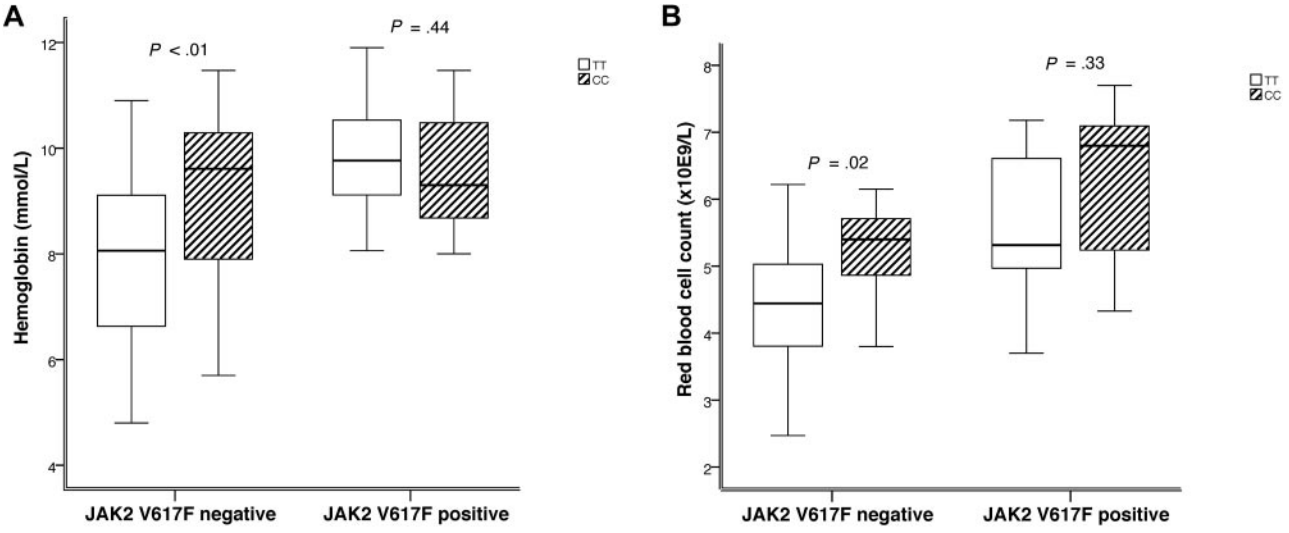

Figure 2. Association between the rs12343867 genotype and laboratory characteristics in patients with splanchnic vein thrombosis. This figure depicts the association between the rs12343867 genotype and hemoglobin levels (A) and red blood cell count (B) in patients with splanchnic vein thrombosis who were homozygous carriers of the common allele (TT) or homozygous carriers of 46/1 (CC). In the *JAK2*^{V617F}-negative group, exclusion of the patients with MPN did not fundamentally alter the associations. Boxplots illustrate the 95% range (vertical lines), median (horizontal lines), and interquartile range (boxes). Comparison between hemoglobin levels and red blood cell counts among the haplotypes was performed using the Kruskal-Wallis test.

with recent studies that have convincingly shown an association between 46/1 and the risk of developing *JAK2*^{V617F}-positive MPNs.[12-14] Only one previous study examined the role of 46/1 in SVT, in which only *JAK2*^{V617F}-negative patients were enrolled.[21] In this study, a significantly elevated risk for the occurrence of PVT in homozygous carriers of 46/1, compared with noncarriers, was reported. However, this study had several limitations. The 46/1 genotype distribution in the control group of this study differs from that of data provided by HapMap-CEU and the WTCCC population.[20] Most notably, the frequency of controls homozygous for the 46/1 was relatively low (2.8%) compared with the HapMap-CEU (6.0%) and WTCCC population (6.5%). In fact, the control group of the study by Colaizzo et al was not in Hardy-Weinberg equilibrium. This has important implications, especially because the reported associations were for homozygous carriers of 46/1 compared with noncarriers. In our study, both controls and patients are representative samples from multiple European countries. This is supported by our control group being in Hardy-Weinberg equilibrium and distribution of 46/1 being consistent with HapMap-CEU and the WTCCC population. We therefore believe our results on the association between 46/1 and PVT are valid and representative for the European population. The increased frequency of 46/1 was observed in *JAK2*^{V617F}-positive SVT patients with a mutant allele burden $< 20\%$ as well as in patients with a *JAK2*^{V617F} allele burden $> 20\%$.

Interestingly, the frequency of 46/1 in *JAK2*^{V617F}-negative SVT patients with a proven MPN was higher compared with the controls (46% vs 27%), although not significantly ($P = .06$). Although we are aware that this group of patients is small, this finding is consistent with the increasing evidence that 46/1 is associated with both *JAK2*^{V617F}-positive and *JAK2*^{V617F}-negative MPNs.[15-17]

An obvious question is whether 46/1 is of additional value in the diagnostic work-up of SVT patients. Our study suggests that 46/1 may be used as a diagnostic tool in the risk assessment of MPNs in SVT patients in addition to the *JAK2*^{V617F} mutation. In a potential diagnostic algorithm, *JAK2*^{V617F} clearly is the first tool to screen for MPNs in SVT patients—the presence of the *JAK2*^{V617F} mutation is highly suggestive, if not pathognomonic, for MPNs in these patients. However, also *JAK2*^{V617F}-negative SVT patients may fulfill the criteria for MPNs. Our study shows that in this specific subgroup of *JAK2*^{V617F}-negative SVT patients, homozygous carriers of 46/1 had a 4- to 5-fold higher chance of MPNs compared with heterozygous carriers or noncarriers. It can be argued that a more aggressive search for MPNs must be instituted in these patients. A bone marrow biopsy is quintessential in these patients, but it might also be considered to intensify the screening program for MPNs during follow-up in these patients. This may facilitate timely recognition and treatment of underlying MPNs. Additional studies are needed to extend our observations before definite conclusions on the potential role of 46/1 in the work-up of SVT patients can be drawn.

We observed a strong association between *JAK2*^{V617F} positivity and clinical and laboratory characteristics at the moment of diagnosis of SVT, which is in line with previous reports on the association between *JAK2*^{V617F} and elevated peripheral blood cell counts,[8,22-25] and that between *JAK2*^{V617F} and splenomegaly.[8,23,24] One of these studies also demonstrated an association between *JAK2*^{V617F} and altered liver function tests and unfavorable prognostic scores in BCS.[8] In the present study we confirm these findings.

The significant association between homozygous carriers of 46/1 and increased erythropoiesis in *JAK2*^{V617F}-negative SVT patients was unexpected. Hemoglobin, hematocrit, and red blood cell count were all approximately 20% higher compared with individuals with the TT genotype. Also, we observed a higher prevalence of splenomegaly in *JAK2*^{V617F}-positive patients homozygous for 46/1. This is the first study to demonstrate an association between 46/1 and laboratory and clinical characteristics. Up to this point, 4 studies examined this association earlier, in which no significant association was observed.[15-18] One study examined the association between 46/1 and the number of granulocyte-macrophage colony forming units (CFU-GMs) in peripheral blood.[12] Carriers of 46/1 grew significantly fewer CFU-GMs, consistent with the hypothesis that 46/1 might be functionally different from other *JAK2*

alleles. Our findings of an increased erythropoiesis support the theory that 46/1 indeed might be functionally different. Theoretically, it is possible that this is specific for SVT patients, especially in light of the unique relationship between MPN and SVT. Clearly, these findings deserve further research.

In conclusion, we observed a clear association between the 46/1 haplotype and the development of *JAK2*^{V617F}-positive SVT. This study also provides the first support of an increased frequency of 46/1 in *JAK2*^{V617F}-negative SVT patients with a proven MPN, and suggests a potential role for 46/1 as a diagnostic tool in SVT in addition to *JAK2*^{V617F}. Unexpectedly, 46/1 was associated with an elevated erythropoiesis in *JAK2*^{V617F}-negative SVT patients, indicating that the 46/1 allele might be functionally different from other alleles.

Acknowledgments

The authors thank all participating physicians for the recruitment of patients and their cooperation with the EN-Vie study; and Joyce Malfliet for her assistance in *JAK2* 46/1 genotyping.

The EN-Vie project was supported by the Fifth Framework Program of the European Commission (contract number QLG1-CT-2002–01 686). S.D.M. is a member of the Mosaic Program of the Netherlands Organization for Scientific Research. Ciberehd is supported by Instituto de Salud Carlos III.

Authorship

Contribution: J.H.S. analyzed the data and wrote the paper; E.K. assisted with data analysis and wrote the paper; S.D.M., A.P., and S.S. collected patient data and samples from patients and controls and coordinated the data organization; J.T. and M.P. collected patient data and samples from patients and controls; M.P.M.d.M. designed the study and assisted with data analysis; J.-C.G.-P., D.C.V, and H.L.A.J. designed the EN-Vie study and coordinated the multicenter collaboration; and F.W.G.L. assisted with analysis of results and wrote the paper.

Conflict of interest disclosure: The authors declare no competing financial interests.

A complete list of all members of the EN-Vie Study Group appears in the supplemental Appendix.

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