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Splanchnic vein thrombosis in myeloproliferative neoplasms: pathophysiology and molecular mechanisms of disease

Joan How, Amy Zhou and Stephen T. Oh

Abstract: Myeloproliferative neoplasms (MPNs) are the most common underlying prothrombotic disorder found in patients with splanchnic vein thrombosis (SVT). Clinical risk factors for MPN-associated SVTs include younger age, female sex, concomitant hypercoagulable disorders, and the *JAK2* V617F mutation. These risk factors are distinct from those associated with arterial or deep venous thrombosis (DVT) in MPN patients, suggesting disparate disease mechanisms. The pathophysiology of SVT is thought to derive from local interactions between activated blood cells and the unique splanchnic endothelial environment. Other mutations commonly found in MPNs, including *CALR* and *MPL*, are rare in MPN-associated SVT. The purpose of this article is to review the clinical and molecular risk factors for MPN-associated SVT, with particular focus on the possible mechanisms of SVT formation in MPN patients.

Keywords: Budd-Chiari syndrome, essential thrombocythemia, *JAK2*, myeloproliferative neoplasm, polycythemia vera, portal vein thrombosis, primary myelofibrosis, splanchnic vein thrombosis

Introduction

Myeloproliferative neoplasms (MPNs) are a group of hematopoietic stem cell disorders characterized by clonal proliferation of myeloid-lineage cells. Classic MPNs include polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and chronic myeloid leukemia (CML) [Tefferi and Vardiman, 2008]. CML is defined by a reciprocal translocation of chromosomes 9 and 22, resulting in the Philadelphia chromosome (Ph), and will not be discussed in this article. The Ph-negative MPNs, which include PV, ET, and PMF, share many common clinical and molecular characteristics, including increased risk of thrombosis and leukemic transformation.

Arterial and venous thromboses are a major cause of morbidity in Ph-negative MPNs. Venous thrombosis may occur at unusual anatomic sites, such as splanchnic vein thrombosis (SVT) or cerebral

sinus thrombosis. SVT refers to thrombosis formation in the portal venous system (portal vein thrombosis, PVT), hepatic venous system Budd-Chiari syndrome (BCS), splenic venous system, or mesenteric venous system. SVTs are rare, with large epidemiological studies estimating the incidence rate to be 0.7 per 100,000 patients per year for PVTs and 0.8 per million patients per year for BCS [Rajani and Almer, 2009; Rajani *et al.* 2010]. This is significantly lower than the incidence rate of deep venous thromboses (DVTs), which is estimated to be roughly 100 per 100,000 patients per year in the United States [White, 2003]. Interestingly, MPNs are the most common underlying prothrombotic disorder found in patients diagnosed with SVT, in the absence of local inciting factors such as liver cirrhosis or nearby malignancy. In patients with BCS specifically, systemic factors, such as an underlying MPN, are more common than local factors [Ageno *et al.* 2014]. The strong association between SVT and MPN

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has led to recommendations to screen for MPN when SVT is diagnosed [Smalberg *et al.* 2012].

The reason for the association between SVT and MPN is not immediately clear. Age, sex, concomitant hypercoagulable disorders, and the presence of the *JAK2* V617F mutation have all been implicated in the pathogenesis of SVT in MPN. Improving our understanding of the mechanisms that predispose to SVT formation is an area of ongoing research. The purpose of this article is to review the clinical and molecular risk factors for MPN-associated SVT, with particular focus on the possible disease mechanisms of SVT formation in MPN patients. The treatment and management of MPN-associated SVTs have been discussed extensively in two recently published reviews [Sekhar *et al.* 2013; De Stefano *et al.* 2015], and will not be discussed in this review.

Prevalence of SVT in MPNs

The prevalence of a venous thromboembolism (VTE) at the time of MPN diagnosis is estimated to be approximately 11–39% for PV, 8–29% for ET, and 3–7% for PMF [Barbui *et al.* 2010; Kreher *et al.* 2014]. SVTs represent a fraction of VTE in MPNs, with an estimated prevalence of 5–10% in PV patients and 9–13% in ET patients [Anger *et al.* 1989; De Stefano *et al.* 2008]. SVTs occur even less frequently in PMF patients, with an estimated prevalence rate of 0.6–1% [Anger *et al.* 1989; Barbui *et al.* 2010]. PVT is the most common type of SVT (40%), while BCS is the least common (5%) [De Stefano *et al.* 2008; Smalberg *et al.* 2012]. Conversely, in patients presenting with SVT, MPNs are the most common underlying prothrombotic disorder. Prevalence rates of MPN in SVT have ranged from 5–70%, with a large meta-analysis estimating that 40% of BCS patients and 30% of PVT patients are subsequently found to have an underlying MPN [Smalberg *et al.* 2012; Sekhar *et al.* 2013]. PV makes up the largest subgroup in patients with SVT (27%), and PMF makes up the smallest subgroup (13%) [Smalberg *et al.* 2012]. Table 1 summarizes the prevalence rates of SVTs in MPNs, and *vice versa*.

Clinical factors associated with SVT in MPN

The proportion of SVT among all VTEs is disproportionately higher in MPN patients compared with the general population, as SVTs make up an estimated 7.5% of total VTE cases [De Stefano *et al.* 2008]. In comparison, the frequency

Table 1. (a) SVTs in patients with MPNs compared with the general population. (b) Prevalence rates of MPNs in patients found to have an SVT.

(a) Prevalence rates of SVTs.		
	Within MPNs	Within general population
PVT	6100 per 100,000	3.7 per 100,000
BCS	1200 per 100,000	0.14 per 100,000
(b) Prevalence rates of MPNs		
	Within PVT	Within BCS
	40%	30%

Sources: De Stefano *et al.* [2008] - 6100/100,000 PVT within MPN; Anger *et al.* [1989] - 1200/100,000 BCS within MPN; Rajani and Almer [2009] - 0.14 per 100,000 BCS within general population; Rajani *et al.* [2010] - 3.7 per 100,000 PVT within general population; Smalberg *et al.* [2012] - prevalence rates of MPNs within PVT and BCS. BCS, Budd-Chiari syndrome; MPN, myeloproliferative neoplasms; PVT, portal vein thrombosis; SVT, splanchnic vein thrombosis.

of DVTs and PEs in the general population is 400 times greater than that of SVTs [Deitelzweig *et al.* 2011]. Interestingly, the risk factors associated with these unusual thromboses are different from the risk factors traditionally associated with all-cause arterial and venous thrombosis. Figure 1 compares the known clinical risk factors of SVTs with those of all-cause VTE.

Age and sex. The best established risk factors for thrombosis in MPN are age >60 years and prior history of thrombosis [Marchioli *et al.* 2005; Kreher *et al.* 2014; Tefferi and Barbui, 2015]. Analysis of 1638 PV patients collected in the ECLAP (European Collaboration on Low-dose Aspirin in Polycythemia vera) trial showed that only advanced age (>60 years), previous venous thrombosis (but not arterial thrombosis), and intermittent claudication demonstrated a statistically significant increased risk of a subsequent VTE [Landolfi *et al.* 2007]. In a multivariate analysis of 891 patients with ET, male sex was also found to be associated with increased venous thrombosis [Carobbio *et al.* 2011]. However, sex was not found to have a significant effect on VTE in PV patients, nor has this finding been demonstrated in other epidemiological studies [Marchioli *et al.* 2005; Landolfi *et al.* 2007].

In contrast, MPN patients with SVTs are predominantly younger and female. Lussana and colleagues evaluated the prevalence of thrombosis in *JAK2*-mutated patients younger than 40 years

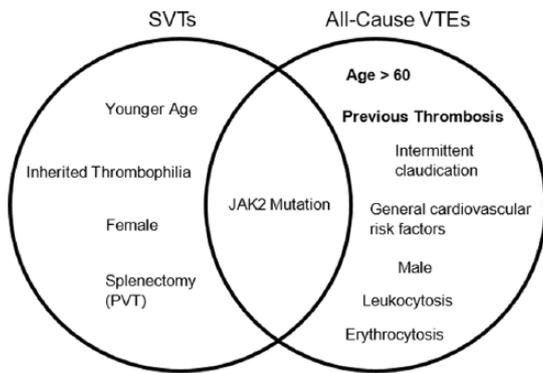


Figure 1. Venn diagram comparing cited clinical risk factors for SVTs and all-cause VTEs. Bold indicates the most validated risk factors.

PVT, portal vein thrombosis; SVT, splanchnic vein thrombosis; VTE, venous thromboembolism.

of age. In this cohort of 538 patients, venous thrombosis was significantly higher than arterial thrombosis (47% *versus* 34%), and SVTs made up 79% (26 out of 33) of these VTE cases [Lussana *et al.* 2014]. This is in contrast with the observation that arterial thromboses rates are 2–3 times higher than venous thrombosis rates in the total MPN population [Barbui *et al.* 2013]. In addition, the proportion of SVTs in this younger population was much higher than in the total MPN population (79% compared with 7.5%) [De Stefano *et al.* 2011; Lussana *et al.* 2014]. Another study, by Stein and colleagues, compared disease characteristics in a cohort of younger (age < 45 years) and older (age > 65 years) MPN patients. The investigators found that although younger patients had a comparable rate of all-cause thrombosis, SVTs occurred significantly more frequently (13% compared with 2%) in patients younger than 45 years of age [Stein *et al.* 2013]. The younger cohort was also predominantly female, and had a significantly lower *JAK2* allele burden [Stein *et al.* 2010, 2013]. The observation that SVTs are significantly more frequent in women compared with men was also noted in the study by Lussana and colleagues [Lussana *et al.* 2014]. There are limited data to suggest that other clinical or laboratory risk factors, such as a history of prior thrombosis or elevated cell counts, increase the risk of SVTs.

Thrombophilic disorders. Hypercoagulable disorders such as antiphospholipid syndrome, protein C or S deficiency, factor V Leiden mutation, or antithrombin deficiency are independent risk

factors for SVT [Kiladjian *et al.* 2008]. In one study of 40 patients with SVTs, it was found that 38% of patients had an additional risk factor to MPN, including a hypercoagulable disorder, paroxysmal nocturnal hemoglobinuria (PNH), autoimmune disorder (such as a connective tissue disease), or ulcerative colitis [De Stefano *et al.* 2011]. Overall, it has been estimated that multiple concurrent risk factors are present in 10–46% of BCS patients and 10–64% of PVT patients [De Stefano *et al.* 2011].

Environmental risk factors. Oral contraceptive (OCP) use has been implicated as a predisposing risk factor for VTEs and cerebral sinus thrombosis [Vandenbroucke *et al.* 1994; Stam, 2005]. It is unclear of the extent to which OCP use increases the risk of SVT formation, given the widespread use of OCPs and the presence of concomitant hypercoagulable disorders. In one study examining the etiological factors in BCS, Smalberg and colleagues identified OCP use in 52% of the 26 female patients. However, these patients often had an additional prothrombotic disorder, and the authors did not specify which combination of etiological factors were present [Smalberg *et al.* 2006]. An additional study examining OCP use and VTE found that nine OCP-users had developed an SVT, of which two were *JAK2* positive [Grandone *et al.* 2008]. Neither of these two women had a concomitant hypercoagulable disorder, although a thrombophilia was present in five of the remaining *JAK2*-negative patients [Grandone *et al.* 2008]. OCP use has been shown to increase the risk of VTE in the presence of an inherited thrombophilia, and it is possible that SVT risk may be similarly increased in MPN patients who use OCPs [Vandenbroucke *et al.* 1994]. This may also partially explain the observation that females are more likely to develop an MPN-associated SVT; however, larger epidemiological studies are needed to potentially establish an association between MPNs, SVTs, and OCP use.

There are few data to suggest that other environmental exposures play a significant role in MPN-associated SVT. Some research has suggested that smoking, along with other general cardiovascular risk factors, increases the rate of arterial thrombosis, but the data are conflicting [Falanga and Marchetti, 2014]. There are few data, however, regarding whether these risk factors apply to venous thrombosis, and specifically SVT.

The *JAK2* V617F mutation in MPN-associated SVT

JAK2 is a tyrosine kinase that initiates intracellular signaling in the *JAK/STAT* pathway *via* binding to receptors for erythropoietin, thrombopoietin, granulocyte colony-stimulating factor, and granulocyte macrophage colony-stimulating factor [James, 2008; Oh and Gotlib, 2010]. The *JAK2* V617F mutation causes constitutive activation of the *JAK2* kinase, resulting in subsequent phosphorylation of *STAT* proteins, ultimately leading to overproduction of myeloid-lineage cells [Campbell and Green, 2006; Oh and Gotlib, 2010]. The identification of the *JAK2* V617F mutation in nearly 95% of PV cases and 50–60% of ET and PMF cases has led to its incorporation into the diagnostic evaluation of MPNs [Campbell and Green, 2006]. Current guidelines recommend testing for *JAK2* mutations in any patient suspected to have an MPN [Ageno *et al.* 2016].

JAK2 mutation in SVT in the absence of overt MPN

An estimated 40% of BCS patients and 28% of PVT patients also test positive for the *JAK2* V617F mutation [Smalberg *et al.* 2012]. For this reason, in any patient presenting with an SVT, *JAK2* testing is recommended as part of the initial work-up for MPN. This has led to the observation that a significant proportion of SVT patients (15–17%) test positive for *JAK2* V617F, but do not otherwise meet WHO criteria for an MPN [Smalberg *et al.* 2012]. Indeed, one study estimated that testing for *JAK2* can identify an additional 30% of MPN patients who do not otherwise have overt evidence of disease [De Stefano *et al.* 2007]. The most frequently cited reason for the absence of elevated blood counts is the presence of portal hypertension and splenomegaly that result from SVT and MPN, which can lead to sequestration of blood cells as well as hemodilution, thus ‘masking’ the clinical phenotype of MPN. Investigators have found that while ‘masked’ patients display lower hemoglobin levels, they also have increased plasma volumes secondary to splenomegaly or SVT formation [Lamy *et al.* 1997].

It is also possible that SVT patients with *JAK2* V617F, but no overt clinical features of MPN, may represent a distinct subtype of MPN or an early phase of the disease. Up to 70% of patients presenting with SVT do not carry a prior diagnosis of MPN, and it has been estimated that SVTs

represent the first thrombotic event in 7.5% of patients with PV and ET [De Stefano *et al.* 2008; Hoekstra *et al.* 2011]. SVTs may thus occur during an early stage of MPN, prior to the development of the clinical phenotype. For instance, one meta-analysis found that 21 out of 41 *JAK2*-positive patients without an MPN diagnosis at the time of SVT presentation subsequently developed an MPN during follow up [Dentali *et al.* 2009]. A more recent study looking at long-term outcomes of SVT patients also observed that 9 out of 13 *JAK2*-positive patients were found to have an MPN after 64 months [Colaizzo *et al.* 2013]. These findings suggest that in these patients, SVTs represent an early manifestation of their disease. In Colaizzo and colleagues’ study, six out of the eight SVT patients who were initially *JAK2*-negative were found to be *JAK2*-positive with clinical symptoms of an MPN by 21 months [Colaizzo *et al.* 2013]. Such observations suggest either that a disease process was already in existence before the occurrence of the *JAK2* mutation, or that the initial *JAK2* V617F allele burden had not reached a detectable level by conventional laboratory testing at the time of SVT diagnosis. The hypothesis that SVTs are early manifestations of MPN may partially account for the finding that MPN-associated SVT patients tend to be younger with lower *JAK2* V617F allele burdens.

The hypothesis that SVT patients represent an earlier stage of MPN disease has important implications in evaluating the underlying molecular pathogenesis of MPNs. It has been hypothesized that blood cancers arise from stepwise genetic mutations that confer survival and growth advantages to hematopoietic stem cells, eventually resulting in clonal hematopoiesis and malignancy [Xie *et al.* 2014]. While early mutations may allow clonal expansion, cooperating mutations that occur later enable development of a hematopoietic cancer. *JAK2*-positive patients without clinical findings of an MPN may lack these cooperating mutations that result in blood cell proliferation. For instance, a significant percentage of MPN-associated mutations, including *JAK2* V617F, have been found in elderly individuals without overt evidence of an MPN [Genovese *et al.* 2014; Xie *et al.* 2014]. These individuals, however, are at an increased risk of developing a subsequent hematologic malignancy and cardiovascular mortality [Jaiswal *et al.* 2014]. These data suggest that mutations such as *JAK2* V617F can be initiating events that may ultimately lead to the development of overt malignancy. Similarly, in SVT

patients who subsequently develop MPNs, it is possible that the development of thrombosis reflects pathology related to an initiating clone. The progression to overt MPN, including elevated cell counts, may only become apparent upon acquisition of subsequent mutations. These patients with positive *JAK2* mutation status may thus be considered a ‘pre-malignant’ state, and as such represent a unique population for studying the clonal evolution of MPNs.

Prevalence of the JAK2 V617F in MPN-associated SVTs

There is a high prevalence of the *JAK2* V617F mutation found in SVT, and the percentage is even higher when restricted to patients meeting MPN criteria with SVT [Patel *et al.* 2006]. An estimated 71–100% of patients with overt MPN and SVT test positive for *JAK2* V617F, which is significantly higher than the prevalence of the *JAK2* mutation in MPN patients with other VTEs [Austin and Lambert, 2008]. A systematic review of 24 studies and 3123 patients found that *JAK2* V617F conferred a significantly increased risk of SVT formation, with an odds ratio of 54 [Dentali *et al.* 2009]. In addition, while the mean prevalence of the *JAK2* mutation in SVTs was high (33%, similar to rates reported in the literature), the mean prevalence of *JAK2* V617F in other VTEs was low (0.88–2.57%) [Dentali *et al.* 2009]. Despite these associations, one study observed that young females tended to have a lower *JAK2* mutant allele burden, even though they are at higher risk for SVTs [Stein *et al.* 2010]. The findings of increased incidence of the *JAK2* mutation but lower allele burden may suggest an ‘all or nothing’ effect of the *JAK2* mutation, which is perhaps subsequently modified by additional clinical risk factors such as concomitant hypercoagulable disorders, as discussed in the previous section.

JAK2 V617F mutation as a molecular driver of SVT formation

The majority of SVTs (with the exception of BCS) are due to local perturbations in the venous system, such as cirrhosis or nearby malignancy. It is possible that the *JAK2* mutation itself may have local effects on the splanchnic venous system. This venous system is unique in that blood flow velocity is much slower, leading to prolonged interactions between blood and sinusoidal endothelial cells [Aird, 2007a]. *In vitro* models have shown that PV

red blood cells demonstrate increased adhesion to endothelial laminin, and this adhesiveness is particularly increased at low shear rates [Wautier and Wautier, 2013]. Furthermore, the portal venous system is exposed to gut-derived inputs, altering its immunogenicity such that vessels are more vulnerable to activated platelets and states of high viscosity such as increased cell count [Aird, 2007b]. These characteristics may put the splanchnic venous system at particular risk to perturbations caused by *JAK2* V617F, which affect not only cell quantity, but cell quality.

The *JAK2* mutation has a widespread effect on the hematologic system. *JAK2*-mutant megakaryocytes display hypersensitive signaling and increased mobility and aggregation [Hobbs *et al.* 2013]. Studies with *JAK2* V617F knock-in mice have demonstrated that megakaryocytes carrying the *JAK2* mutation show greater chemotaxis along migration assays, as well as increased pro-platelet formation in response to low-level thrombopoietin signaling [Hobbs *et al.* 2013]. *JAK2*-positive platelets also display increased *in vitro* thrombus formation when exposed to collagen-coated surfaces, although Lamrani and colleagues found decreased platelet reactivity when tested in a different mouse model [Hobbs *et al.* 2013; Lamrani *et al.* 2014]. However, accelerated platelet aggregation occurred when studied *in vivo* in artificially injured blood vessels [Lamrani *et al.* 2014]. Blood samples taken from *JAK2*-positive patients with MPN also show significantly higher levels of soluble P-selectin, a marker of platelet activation, compared with *JAK2*-negative patients with MPN [Robertson *et al.* 2007].

Leukocyte activation also contributes to the pro-coagulant milieu, as studies have shown that markers of polymorphonuclear activation correlate with markers of hypercoagulability in ET and PV patients [Falanga *et al.* 2005]. It is thus believed that activated leukocytes can cause secretion of pro-coagulant factors, leading to further platelet activation and aggregation. In *JAK2*-positive patients, the level of both leukocyte and platelet activation increases with higher *JAK2* mutational burden, and an epidemiological study by Barbui and colleagues found that the highest incidence of thrombosis was seen in PMF patients with both leukocytosis and the *JAK2* mutation [Arellano-Rodrigo *et al.* 2006; Barbui *et al.* 2013]. Red blood cells also display increased adhesion to the endothelium in PV patients [Wautier and Wautier, 2013]. This effect may be mediated by

aberrations in JAK/STAT signaling, as *JAK2* V617F can induce phosphorylation of glycoproteins critical for red blood cell adhesion [De Grandis *et al.* 2013]. Taken together, these findings suggest that the *JAK2* mutation leads to distinct changes in hematopoietic cells that increase the likelihood of thrombus formation.

The propensity for SVT development in MPN is also potentially due to differential regulation of hemostasis in specific vascular beds. Endothelial cells are integrally involved in regulating thrombosis, and are capable of expressing both pro- and anticoagulant factors depending on both the environmental milieu and cell type [Rosenberg and Aird, 1999]. This is supported by observations that MPNs are associated with specific anatomical locations within the splanchnic venous system. In MPN patients with BCS, for example, thrombosis tends to occur in the large, hepatic veins, while BCS patients with other hypercoagulable disorders tend to have thrombosis involving the inferior vena cava [Valla, 2015]. Signaling pathways specific to the splanchnic venous system can then cause unique interactions with mutated blood cells. For instance, in PNH, a complement-mediated disorder that also presents with increased susceptibility to SVT, the presence of gut-derived antigens in the splanchnic veins may cause higher local activation of complement, with subsequent higher rates of hemolysis and thrombotic activation [van Bijnen *et al.* 2012].

Mutant endothelial cells may also contribute to SVT pathogenesis. Liver endothelial cells isolated from two BCS patients have been found to carry the *JAK2* mutation [Sozer *et al.* 2009]. Further studies have identified the *JAK2* mutation in spleen endothelial cells and circulating endothelial progenitor cells [Teofili *et al.* 2011; Rosti *et al.* 2013]. *JAK2* V617F-mutated endothelial progenitor cells also displayed increased adherence to normal mononuclear cells, and were found to abnormally activate the JAK/STAT pathway [Teofili *et al.* 2011]. These findings provide a framework in which potentially aberrant endothelial cells in the splanchnic venous system interact with aberrant blood cells, eventually leading to the formation of SVT. The exact mechanism of how alterations in the endothelium lead to SVT is still unclear, and warrants further investigation. Given the unique environment of the splanchnic venous system, it is likely that the mechanisms of SVT formation differ from the mechanisms of

arterial and DVT, accounting for the difference in risk factors.

Other molecular drivers of MPN-associated SVT

Although the *JAK2* mutation plays a significant role in MPN in SVT, approximately 14–20% of SVT patients with MPN are *JAK2*-negative [Kiladjian *et al.* 2008; Smalberg *et al.* 2012]. In terms of clinical risk factors, *JAK2*-negative patients with MPN/SVT are not statistically different from their *JAK2*-positive counterparts [Kiladjian *et al.* 2008]. This leads to the question of whether there are other mutations that can account for the prothrombotic state. Specifically, several mutations with increased incidence in MPN have been identified, and only recently have investigators evaluated these mutations in SVT.

CALR mutations

The *CALR* gene encodes for calreticulin, a multifunctional protein that regulates calcium signaling and protein folding in the endoplasmic reticulum and cytoplasm [Johnson *et al.* 2001]. Only recently has a mechanism for *CALR*-mutant MPN pathogenesis been proposed. A series of papers demonstrated that mutated calreticulin is able to induce MPN phenotypes in mice through interaction with the thrombopoietin receptor (MPL) [Araki *et al.* 2016; Chachoua *et al.* 2016; Elf *et al.* 2016; Marty *et al.* 2016]. Mutated calreticulin has been shown to bind preferentially to MPL, resulting in thrombopoietin-independent activation of MPL with subsequent stimulation of the JAK/STAT pathway [Araki *et al.* 2016; Chachoua *et al.* 2016; Elf *et al.* 2016; Marty *et al.* 2016]. The specificity of mutant calreticulin interaction with MPL accounts for the findings that *CALR* mutations are largely absent in PV, but found in 30% of all ET and PMF patients, and 70–80% of *JAK2*-negative ET or PMF [Klampfl *et al.* 2013; Nangalia *et al.* 2013]. Compared with *JAK2*, *CALR*-mutant patients tend to have increased platelet counts, lower hemoglobin and white cell counts, as well as decreased rates of thrombosis, with a more indolent disease course [Nangalia *et al.* 2013].

How or whether *CALR* mutations induce SVT susceptibility is less clear. Despite the relatively high frequency of *CALR* mutations in MPNs, recent studies have failed to find an increased incidence of *CALR* mutations in SVT patients.

CALR mutations have been identified in 0–2% of SVT, with an estimated total prevalence of approximately 0.9% of cases [Castro *et al.* 2015; Colaizzo *et al.* 2015; Haslam and Langabeer, 2015; Iurlo *et al.* 2015; Marzac *et al.* 2015; Plompen *et al.* 2015; Roques *et al.* 2015; Turon *et al.* 2015]. Given its relatively low prevalence, testing for *CALR* is not generally recommended in patients with SVT [Colaizzo *et al.* 2015]. Furthermore, its rarity suggests that it is not a major contributor to SVT formation, a fact consistent with the observation that *CALR*-mutated patients exhibit decreased rates of thrombosis overall [Rumi *et al.* 2014].

MPL mutations

Mutations in the *MPL* gene are the third most commonly tested for mutation after *JAK2* and *CALR* in MPN patients. *MPL* encodes for the thrombopoietin receptor, and mutations expectedly cause abnormalities in megakaryocyte and platelet production. The most common mutations result in an amino acid substitution (either lysine or leucine) at the 515 position (*MPL* W515), are found in approximately 5% of ET/PMF patients, and are mutually exclusive to *JAK2* and *CALR* mutations [Pikman *et al.* 2006; Rumi *et al.* 2013]. *MPL*-mutant patients tend to be older, with lower cell counts compared with *JAK2* and *CALR*-positive patients [Beer *et al.* 2008]. *MPL* mutations have not been shown to confer significant prognostic impact, and patients do not have differing risks of thrombosis or major hemorrhage [Beer *et al.* 2008]. However, similar to *CALR*, *MPL* W515 mutations are rare in SVT, with incidence rates even less than *CALR* [Bergamaschi *et al.* 2008; Kiladjian *et al.* 2008; Jadli *et al.* 2012; Smalberg *et al.* 2012; Colaizzo *et al.* 2015]. This may be related to the overall low prevalence of *MPL* in MPNs, but there is no evidence to suggest that *MPL*-positive patients have higher rates of SVTs.

The JAK2 46/1 haplotype

The *JAK2* 46/1 haplotype has recently been found to be associated with *JAK2*-positive MPNs, and it is believed that the *JAK2* V617F mutation arises specifically on the 46/1 allele [Jones *et al.* 2009]. Its role in SVT formation is controversial, with some studies showing that the haplotype is an independent risk factor for SVT development in *JAK2* V617F patients [Colaizzo *et al.* 2010a; Smalberg *et al.* 2011], and some studies showing

only a weak association [Kouroupi *et al.* 2011]. A recent meta-analysis of 26 observational studies evaluated the risk of MPN with the *JAK2* haplotype, and found that the haplotype was significantly increased in MPN and SVT patients. In *JAK2*-negative patients, however, the haplotype did not confer an additional risk of SVT [Li *et al.* 2014]. The increased risk of SVT with the 46/1 haplotype may be due to an interaction of the haplotype with the *JAK2* V617F mutation, rather than the haplotype itself.

Additional mutations in MPN-associated SVTs

Approximately 10% of MPN patients are ‘triple-negative’ and do not harbor *JAK2*, *CALR*, or *MPL* mutations [Tefferi *et al.* 2014]. Additional mutations are found in varying frequencies in PV, PMF, and ET patients, although they tend not to be mutually exclusive with *JAK2*, *CALR*, *MPL* mutations, or with each other. These mutations include *TET2*, *DNMT3A*, *EZH2* and *ASXL1*, and are involved in DNA methylation and chromatin structuring [Delhommeau *et al.* 2009; Grand *et al.* 2009; Ernst *et al.* 2010; Abdel-Wahab *et al.* 2011]. The specific role of these mutations in driving MPN disease formation is not fully understood. As discussed previously, SVT patients may be an ideal population for investigating the role of precursor genes and initiating mutations, as these patients may present at an early stage of MPN disease evolution. For instance, *TET2* is a tumor suppressor gene which plays a key role in DNA methylation, and mutations in *TET2* have been identified in up to 12% of MPN patients, including both *JAK2*-positive and *JAK2*-negative MPNs [Delhommeau *et al.* 2009]. In the hematopoietic stem cells of patients with both *JAK2* and *TET2* mutations, the *TET2* mutation could often be found in the absence of the *JAK2* mutation, suggesting the acquisition of *TET2* before *JAK2* [Delhommeau *et al.* 2009]. In surveying SVT patients, however, only a minority (3/23) of SVT patients tested positive for *TET2*, while all were *JAK2* positive [Colaizzo *et al.* 2010b]. A larger study of 43 BCS patients identified 6 patients with a deleterious *TET2* mutation; however, only 3 were *JAK2*-negative and none of these 3 patients developed an overt MPN [Westbrook *et al.* 2012]. Based on the studies by Colaizzo and colleagues and Westbrook and colleagues, the prevalence of *TET2* in SVTs is roughly 14%, which is similar to the prevalence rate of

Table 2. Prevalence of MPN-associated mutations in SVT and MPN patients. *JAK2* makes up the highest proportion of patients, while *CALR* and *MPL* have very low prevalence rates in SVTs. *TET2* is found at higher prevalence rates but is not mutually exclusive with *JAK2*.

	SVT patients	MPN patients
<i>JAK2</i>	33%	95% in PV; 60% in ET and PMF
<i>CALR</i>	0.9%	30% in ET and PMF
<i>MPL</i>	0.7%	5% in ET and PMF
Other		
<i>TET2</i>	14%	12%

Sources: *MPL*- 0.7% in SVT, *JAK2*- 33% in SVT patients- Smalberg *et al.* [2012]; *CALR*- 0.9% in SVT patients- De Stefano *et al.* [2015]; *MPL*- 0.7% in SVT- Bergamaschi *et al.* [2008]; *CALR*- 0.9% in SVT patients- Colaizzo *et al.* [2015]; *MPL*- 0.7% in SVT- Kiladjian *et al.* [2008]; *MPL*- 0.7% in SVT, *TET2*- 14% in SVT- Jadli *et al.* [2012]; *CALR*- 0.9% in SVT patients- Turon *et al.* [2015]; *TET2*- 14% in SVT- Westbrook *et al.* [2012]; *TET2*- 14% in SVT- Colaizzo *et al.* [2010b]; 12% in MPN- Delhommeau *et al.* 2009; 5% in ET and PMF - Pikman *et al.* 2006; 95% in PV, 60% in ET/PMF- Campbell and Green 2006; 30% in ET and PMF- Klampf *et al.* 2013.
ET, essential thrombocythemia; MPN, myeloproliferative neoplasms; PMF, primary myelofibrosis; PV, polycythemia vera; SVT, splanchnic vein thrombosis.

TET2 in all MPNs [Delhommeau *et al.* 2009]. While *TET2* is found more frequently than *CALR* and *MPL* in SVT, its frequent co-occurrence with *JAK2* and lower prevalence rate suggests a limited role in SVT formation. Further investigations for mutations present in *JAK2*-negative SVT patients are limited. A small study of 25 cases of *JAK2*-negative BCS found no patients with *IDH1* or *IDH2* mutations [Jadli *et al.* 2012].

Table 2 summarizes the prevalence of the most common MPN-associated mutations in SVTs and all MPNs. In light of the existing literature, there seems to be a lack of evidence to suggest that these other mutations are associated with SVT formation in MPN, despite the finding that 14–20% of SVT patients with an MPN are *JAK2*-negative [Kiladjian *et al.* 2008; Smalberg *et al.* 2012]. The existence of a precursor mutation to *JAK2* V617F critical to SVT pathogenesis has yet to be identified. Additional research examining the clinical and molecular differences between *JAK2*-positive and *JAK2*-negative patients with SVT is needed to further elucidate the driving mechanisms of SVT formation and MPN disease progression.

Future directions and conclusion

The development of SVTs in MPN is multifactorial and likely includes both environmental and host genetic factors. Risk factors for the development of SVT in MPN patients include the *JAK2* V617F mutation, young age, female sex, and the presence of concomitant hypercoagulable disorders. Intrinsic characteristics of the splanchnic venous bed may predispose to thrombotic interactions with atypical blood cells, which are modified by *JAK2* V617F or unknown precursor mutations. Furthermore, the mechanisms that cause SVT formation likely differ from the mechanisms that cause arterial and DVT, accounting for the differences in risk factors. There is little evidence to suggest that other known genetic alterations implicated in MPN pathogenesis, such as *CALR*, *MPL*, or the *JAK2* 46/1 haplotype, are critical for SVT formation. Given that SVT patients may present at an early stage of MPN, identifying common mutations may shed light on the molecular pathogenesis of MPN disease development and progression.

Research into understanding the occurrence of SVTs in MPNs also has important implications for treatment and prognosis. Identifying patients most at risk for SVT can prevent considerable morbidity and mortality. Investigation into the use of *JAK2* inhibitors to decrease the risk of future thrombosis is already under way [Keohane *et al.* 2015]. Future directions for research include better characterization of MPN patients with and without SVTs, and the development of a database of such phenotypes. In particular, investigating differences between *JAK2*-positive and *JAK2*-negative patients can better clarify the role of *JAK2* V617F in thrombosis formation.

In summary, SVTs occur in a unique subset of MPN patients, and often present with little overt MPN disease characteristics. Understanding the association between SVTs and MPNs can provide insight not just on possible mechanisms of thrombosis, but also the pathophysiology of disease evolution.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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