The JAK2 V617F mutation frequently occurs in patients with portal and mesenteric venous thrombosis

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Summary. Background: Myeloproliferative disorders (MPDs) represent a risk factor for thrombosis in the portal, mesenteric, and hepatic districts. Objective: We aimed to assess whether the Janus kinase 2 (JAK2) V617F mutation, an acquired mutation that occurs in MPD patients, is a risk factor for portal and mesenteric venous thrombosis (PMVT) independently of the presence of overt MPDs. Patients and methods: The medical histories of 99 patients presenting with PMVT were obtained. The presence of the JAK2 V617F and VHL 598C > T mutations was determined by polymerase chain reaction followed by restriction enzyme analysis and direct cycle sequence analysis. Results: Over a 10-year period of observation, of the 99 patients presenting with PMVT, the JAK2 V617F mutation was detected in heterozygous state in 17 individuals (17.2%; 95% confidence interval (95% CI) 10.9–25.9). None of the patients presenting with the JAK2 V617F mutation carried an inherited thrombophilic risk factor. Seven patients with (43.8%; 95% CI 19.8–70.1) and two without (2.4%; 95% CI 0.3–8.4) the JAK2 V617F mutation had a diagnosis of MPD at the occurrence of the venous thrombotic event. After a median follow-up of 41 months (range 3–114 months), three out of the 10 patients carrying the JAK2 V617F mutation were then diagnosed as having idiopathic myelofibrosis (n = 2) or polycythemia vera (n = 1), whereas in seven patients a MPD was not detected. Two of the 83 patients without the JAK2 V617F mutation went on to develop MPDs. Conclusions: Determination of the JAK2 V617F mutation may contribute to the search for genetic determinants of PMVT and may be useful to recognize patients who should be carefully observed for the subsequent development of overt MPDs.

Keywords: JAK2, mutation, myeloproliferative disorders, risk factor, venous thrombosis.

Introduction

Venous thrombosis is the third most common cardiovascular affliction after ischemic heart disease and stroke [1]. The pathogenesis of venous thrombosis is multifactorial, involving acquired and genetic factors. In addition to circumstantial predisposing factors (e.g. surgery, pregnancy, immobilization, malignancy), genetic predisposition because of molecular abnormalities of components of the coagulation pathway have been found in subjects who had suffered from thromboembolic disease [2]. Splanchnic venous thrombosis, including thrombosis of the hepatic veins or portal venous system, is an uncommon event and may be caused by a variety of conditions including cirrhosis and cancer, and abdominal infectious and inflammatory processes [3]. Coexistence of causative factors was observed in a large setting of patients, indicating that thrombosis of the portal vein, like other manifestations of thrombosis, can be a result of combined pathogenetic mechanisms.

Myeloproliferative disorders (MPDs), whether overt or latent, represent a main intrinsic factor for the development of thrombosis in the portal, mesenteric, and hepatic areas [4–7]. Venous thrombosis can involve all territories but MPDs, mainly polycythemia vera (PV) and essential thrombocytosis (ET), are the most common underlying etiology for Budd–Chiari syndrome and PMVT; spontaneous endogenous erythroid-colony formation being seen in up to 78% of patients thought to have Budd–Chiari Syndrome and in 48% of patients with splanchnic venous thrombosis [8]. On the other hand, venous thromboses significantly affect morbidity and mortality of patients with MPD and are associated with severe organ damage and high mortality [9].

Recently, several groups identified a recurrent activating tyrosine kinase mutation, Val617Phe, in the JH2 pseudo-kinase domain of the Janus kinase 2 (JAK2) gene in patients with sporadic MPDs. This mutation was found in most patients with PV and in about half of the patients with ET or
myelofibrosis (MF) [10–14]. This JAK2 mutation is an acquired somatic event in sporadic MPDs, leading to a constitutive activation of the JAK–signal transducer and activator of transcription signal transduction pathway [15].

It is well-known that PMVT may be an early or presenting complication of an undiagnosed MPD, particularly in young patients [4,8] and that a portion of these patients do not fulfill the diagnostic criteria for MPD but have features suggestive of a latent form based on hyperplastic bone marrow and erythropoietic progenitor cell culture; these cases may subsequently develop overt MPD. Actually, a meta-analysis of 120 patients from seven studies indicated that the estimated incidence of underlying MPD depends on the diagnostic criteria used [8]. Very recently, the JAK2 V617F mutation has been found to occur in a high proportion of patients with Budd–Chiari syndrome, a heterogeneous group of disorders resulting from obstruction to hepatic venous outflow [16].

The Chuvash polycythemia is associated with specific germline mutations in the von Hippel–Lindau tumor suppressor (VHL) gene, mainly the 598C > T, [17] and is characterized by a higher occurrence of splanchnic venous thrombosis [18,19].

Thus, we have investigated whether the clonal JAK2 V617F mutation and/or the VHL 598C > T mutation occur in a high proportion of non-cirrhotic patients with portal and mesenteric venous thrombosis (PMVT) and whether determination of these mutations would be useful in the characterization of latent MPD in this patient setting.

Materials and methods

Patients and controls

Patients with PMVT consecutively diagnosed and followed-up at Gastroenterology Unit of the ‘A. Cardarelli’ Hospital, Naples were referred to the Thrombosis Centre of the same hospital for a work-up at least 3 months after the thrombotic episode. All subjects with liver cirrhosis, hepatocellular carcinoma, or who reported a personal history of Budd–Chiari syndrome, were excluded from the study. Between January 1997 and June 2006, we investigated 112 non-anticoagulated patients (median age 42 years; range 10–85 years), 50 men and 62 women, with a documented portal and/or mesenteric venous thrombosis. A complete clinical summary, with emphasis on personal and family history of thromboembolic disease and circumstantial vascular risk factors (surgery, immobilization, pregnancy, post-partum, trauma, oral contraception, varicose veins and malignancy), was obtained from all subjects by a specially trained staff. Portal and mesenteric venous thromboses were diagnosed by Doppler ultrasonography, spiral computed tomography, or magnetic resonance imaging as required during the routine diagnostic work-up. MPDs were diagnosed according to established criteria [20].

Two-hundred and thirty apparently healthy subjects (98 men and 132 women; median age 44 years; range 21–73 years) randomly selected from a southern Italian general population of employees of the ‘Casa Sollievo della Sofferenza’ Hospital, S. Giovanni Rotondo, without a history of venous thromboembolism, served as controls. All subjects who reported a personal history of clinical venous thrombosis were excluded from the study. Both cases and controls were Caucasian and were from the same region. The two groups were comparable for sex, social status, and age.

After approval from the local Ethics Committees, the study was carried out according to the Principles of the Declaration of Helsinki. Informed consent was obtained from all the subjects.

Blood collection and coagulation tests

Blood samples were collected into vacuum plastic tubes containing 3.8% trisodium citrate and centrifuged at 2000 × g for 15 min to obtain platelet-poor plasma. After having filtered a portion for the assessment of lupus anticoagulant, samples were frozen and stored in small aliquots at −70 °C until tested. Antiphospholipid antibodies [lupus anticoagulant and immunoglobulin G antcardioplin antibodies (ELISA, Byk Gulden, Italy)], antithrombin, protein C, amilod-lytic and immunological (Behring, Marburg, Germany) and total and free protein S antigen (ELISA, Diagnostica Stago, Asnières, France) were determined in all patients, as reported elsewhere [21]. Clotting assays were performed on a KC4 Amelung coagulometer (Amelung, Germany). Inter- and intra-assay coefficients of all the variables never exceeded 8.0% and 5.0%, respectively.

DNA extraction and analysis

DNA was extracted from peripheral blood leukocytes according to standard protocols [22]. A 220-bp DNA fragment of the factor (F) V gene that includes the nucleotide 1691 was amplified and digested with MnlI as previously described [23]. To identify the G > A mutation of the prothrombin gene, a 345-bp fragment was obtained and then digested using the HindIII endonuclease, as reported [24].

Amplifications of regions of JAK2 gene containing the V617F were performed as previously described [25]. Genomic DNA was amplified by polymerase chain reaction (PCR), and successful amplification was confirmed by electrophoresis on an ethidium bromide-impregnated 2.0% agarose gel. Oligonucleotide custom synthesis service was from Life Technologies (Paisley, UK). PCRs were carried out on 50 μL volume samples, in a Perkin Elmer Cetus thermal cycler (Perkin Elmer Cetus, Norwalk, CT, USA). Each sample contained 0.1 μg of genomic DNA, and 20 pmol L⁻¹ each of sense and antisense primers (5'-AGCAAGCTTTCACAAAGCA-3' and 5'-CTGACACCTAGCTTGATCCTG-3', respectively), 125 μM of dNTP, 5 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, and 1 U Taq polymerase. The solution was overlaid with 50 μL of mineral oil and, after an initial denaturation step (3 min at 95 °C), it was put through 30 cycles, each consisting of 1 min at 95 °C, 1 min at 56–60 °C.
and 2 min at 72 °C. Then, 5 μL volumes of the amplification product were separated in 2% agarose-gel electrophoresis in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 7.7) containing 0.5 μg mL−1 ethidium bromide, and visualized under ultraviolet light. Amplification products were subjected to restriction enzyme analysis using 2 IU of the BsaXI endonuclease (New England Biolabs Inc., Beverly, MA, USA), as previously reported [25]. The G-to-T transversion in exon 12 leads to the missing of a site of digestion of BsaXI, giving rise to an uncut product instead of the two expected restriction fragments (96 and 56 bp). Then, amplified DNA fragments showing an abnormal pattern of digestion were cleaned with a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) and subjected to direct cycle sequence analysis using the Taq dye-deoxy terminator method, the same primers used for amplification, and an ABI PRISM 310 Genetic Analyzer sequencer (PE Biosystems, Foster City, CA, USA). Because the JAK2 V617F mutation is an acquired somatic event, we indirectly assessed the sensitivity of methods employed to detect it using serial dilutions of the amplification product from a patient homozygous for the FV Q506 allele with that from a subject homozygous for the FV R506 allele. Using the restriction enzyme analysis, we were able to detect dilutions as low as 5%, whereas the lower limit of the direct cycle sequence analysis was 20%.

The genotyping of the VHL 598C > T mutation was performed as previously described [17]. The VHL 598C > T mutation was identified by Fnu4HI restriction endonuclease (New England Biolabs) that digests the nucleotide sequence of the wild allele (C), but its targeted restriction site is abolished by the C-to-T transition. Then, amplified DNA fragments showing an abnormal pattern of digestion were cleaned with QIAquick PCR purification kit (Qiagen) and subjected to direct cycle sequence analysis using the Taq dye-deoxy terminator method, the same primers used for amplification, and an ABI PRISM 310 Genetic Analyzer sequencer (PE Biosystems).

Statistical analysis

All analyses were performed according using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). The significance of any difference in means was evaluated by non-parametric test, whereas the significance of any difference in proportions was tested by chi-squared statistics. The allele frequencies were estimated by gene counting, and genotypes were scored. The observed numbers of each gene mutation [Factor V Leiden (FV Leiden), FII A20210, JAK2 V617F, and VHL 598C > T] were analyzed numbers of each gene mutation [Factor V Leiden (FV estimated by gene counting, and genotypes were scored. The tested by chi-squared statistics. The allele frequencies were difference in means was evaluated by non-parametric test, whereas the significance of any difference in proportions was evaluated by non-parametric test. Statistical significance was taken as \( P < 0.05 \).

Results

Description of the cohort

Between January 1997 and June 2006, 112 patients (50 men and 62 women) were diagnosed with documented PMVT. Thirteen patients (11.6%) were not studied: consent was refused in one; DNA samples were not available in four; six were lost in the follow-up; and there were technical problems in two. Thus, we analyzed 99 patients (44 men and 55 women; median age 41 years; range 10–85 years).

Demographic characteristics and the incidence of circumstantial and thrombophilic risk factors both in patients and in controls are shown in Table 1. An acquired thrombophilic risk factor was identified in 38 (38.4%; 95% CI 28.8–48.7) patients. The most represented were: history of a hematological malignancy (\( n = 11 \); MPD in nine and lymphoma in two); previous surgical intervention (\( n = 10 \); and the prescription of oral contraceptives (\( n = 8 \). In five patients a moderate hyperhomocysteinemia was found, and no patient displayed antiphospholipid antibodies.

Patients had a higher incidence of the FII A20210 but not of the FV Leiden mutation. Actually, in this setting, the number of carriers of the FV Leiden mutation was eight (8.1%; 95% CI 3.6–15.3), seven heterozygotes and one homozygote, among patients, and nine heterozygotes (3.9%; 95% CI 1.8–7.3; OR 2.2; 95% CI 0.8–5.6) in healthy individuals. The FII A20210 mutation was detected in 12 cases (12.1%; 95% CI 6.4–20.2), all heterozygotes, and, in the heterozygous state, only in seven controls (3.0%; 95% CI 1.2–6.2; OR 4.4; 95% CI 1.7–11.2). Frequencies of all the mutations were similar in men and women (not shown). One patient was homozygous for the FV Leiden mutation and heterozygous for the FII A20210 mutation. In seven patients the coexistence of acquired and inherited

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (range)</td>
<td>44.0 (21–73)</td>
<td>41.0 (10–85)</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>98 (42.6%)</td>
<td>44 (44.4%)</td>
</tr>
<tr>
<td>Acquired risk factors, n (%)</td>
<td>NA</td>
<td>38 (38.4%)</td>
</tr>
<tr>
<td>Hematologic malignancy, n (%)</td>
<td>NA</td>
<td>11 (11.1%)</td>
</tr>
<tr>
<td>Surgery, n (%)</td>
<td>NA</td>
<td>10 (10.1%)</td>
</tr>
<tr>
<td>Oral contraceptives, n (%)</td>
<td>NA</td>
<td>8 (8.1%)</td>
</tr>
<tr>
<td>Homocysteine &gt; 95 centile, n (%)</td>
<td>NA</td>
<td>5 (5.1%)</td>
</tr>
<tr>
<td>Others, n (%)</td>
<td>NA</td>
<td>8 (8.2%)</td>
</tr>
<tr>
<td>Inherited risk factors, n (%)</td>
<td>NA</td>
<td>21 (21.2%)</td>
</tr>
<tr>
<td>Factor V Leiden, n (%)</td>
<td>9 (3.9)</td>
<td>8 (8.1%)</td>
</tr>
<tr>
<td>Factor II A20210 allele, n (%)</td>
<td>7 (3.0)</td>
<td>12 (12.1%)</td>
</tr>
<tr>
<td>Antithrombin deficiency, n (%)</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Protein C deficiency, n (%)</td>
<td>NA</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Protein S deficiency, n (%)</td>
<td>NA</td>
<td>0</td>
</tr>
<tr>
<td>Risk factors, n (%)</td>
<td>NA</td>
<td>53 (53.5%)</td>
</tr>
<tr>
<td>Combined risk factors, n (%)</td>
<td>NA</td>
<td>12 (12.1%)</td>
</tr>
</tbody>
</table>

*Four patients carried combined acquired risk factors; 1 patient carried combined inherited risk factors. ^OR 2.2; 95% CI 0.8–5.6; 3 OR 4.4; 95% CI 1.7–11.2. NA, not applicable.
thrombophilic risk factors was observed. Two additional patients had an isolated protein C deficiency. As a whole, a thrombophilic risk factor was identified in 53 patients (53.5%; 95% CI 43.2–63.6) and in 12 of them (12.1%; 95% CI 6.4–20.2) more than one risk factor was recorded. The median age of patients carrying a thrombophilic risk factor was 42 years (range 15–80 years) and was similar to that of patients without thrombophilic risk factors (40.0 years; range 10–72 years) \((P = 0.612;\) Mann–Whitney U-test).

**JAK2 V617F mutation and the occurrence of PMVT**

Among patients with PMVT, 17 (17.2%; 95% CI 10.9–25.9), five men and 12 women, carried the JAK2 V617F mutation (Table 2); all were heterozygotes. No members of the control group were found to carry the mutant allele. Samples from all subjects with PMVT and carrying the JAK2 V617F mutation were sequenced. In 16 out of 17 patients, the sequencing analysis showed a concordance with results obtained with the restriction fragment length analysis. In these patients, allele quantization, estimated taking into account the relative area of both alleles, gave comparable results for the wild (G) and the mutant (T) allele, the ratio never exceeding 60%. In the remaining patient, a 28-year-old woman, the mutant allele was detected only by restriction enzyme analysis. The median age of positive patients was 44 years (range 28–85 years) and was slightly higher than that (39.5 years; range 10–72 years) of the 82 (82.8%; 95% CI 74.1–89.1) patients, 39 men and 43 women, without the JAK2 V617F mutation (0.195; Mann–Whitney U-test). None of patients presenting with the JAK2 V617F mutation carried an inherited thrombophilic risk factor, such as FV Leiden, FII A20210 mutation, or protein C deficiency. The knowledge of a MPD at the occurrence of the venous thrombotic event was recorded in seven (41.2%; 95% CI 18.4–67.1) patients with and two (2.4%; 95% CI 0.3–8.5) without \((P = 0.012;\) two-tailed Fisher’s exact test). However, after excluding MPD, the difference was no longer significant, other acquired risk factors being found in only four patients (Table 2). Overall, the JAK2 V617F mutation was detected in seven out of nine patients (77.8%; 95% CI 40.0–97.2) presenting with a known MPD, as shown by peripheral blood counts and bone marrow biopsy. In detail, three patients with idiopathic MF, two out of three with ET, one out of two with PV, and one woman with chronic myeloid leukemia showed the JAK2 V617F mutation.

**VHL 598 C > T mutation and the occurrence of PMVT**

Among patients with PMVT, two (2.0%; 95% CI 0.3–7.1), a 38-year-old man and a 28-year-old woman, carried the VHL 598C > T mutation; both were heterozygotes. The presence of the mutation in a heterozygous condition was confirmed by sequencing analysis. No members of the control group were found to carry the mutant allele \((P = 0.180;\) two-tailed Fisher’s exact test). None of patients presenting with the VHL 598C > T mutation carried an acquired thrombophilic risk factor, whereas the FII A\textsuperscript{20210} mutation was identified in the male patient.

**Follow-up of patients presenting with PMVT**

All patients presenting with PMVT without a known diagnosis of MPD had serial evaluations of hematocrit and hemoglobin, and leukocyte and platelet counts. In addition, a karyotype analysis and the search for the \(bcr-abl\) rearrangement was performed in all subjects. During the follow-up (median 41 months; range 3–114 months), five out of 90 patients (5.6%; 95% CI 1.8–12.5) went on to develop MPDs: two idiopathic MF (one man and one woman), one PV (woman),

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### Table 2 Clinical characteristics of patients carrying the JAK2 V617F mutation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at diagnosis</th>
<th>Year</th>
<th>Myeloproliferative disorders</th>
<th>Risk factors</th>
<th>Vein thromboses</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMP</td>
<td>Woman</td>
<td>49</td>
<td>1997</td>
<td>PV during the work-up</td>
<td>None</td>
<td>Portal, mesenteric</td>
</tr>
<tr>
<td>VC</td>
<td>Woman</td>
<td>61</td>
<td>1997</td>
<td>CML known</td>
<td>Hypohomocysteinemia</td>
<td>Spleen, portal, mesenteric</td>
</tr>
<tr>
<td>CMR</td>
<td>Woman</td>
<td>52</td>
<td>1998</td>
<td>ET known</td>
<td>None</td>
<td>Portal, mesenteric</td>
</tr>
<tr>
<td>CA</td>
<td>Woman</td>
<td>44</td>
<td>2000</td>
<td>MF during follow-up</td>
<td>Abdominal surgery</td>
<td>Portal, mesenteric</td>
</tr>
<tr>
<td>DSMR</td>
<td>Woman</td>
<td>46</td>
<td>2000</td>
<td>No</td>
<td>None</td>
<td>Portal</td>
</tr>
<tr>
<td>LR</td>
<td>Woman</td>
<td>35</td>
<td>2000</td>
<td>MF known</td>
<td>None</td>
<td>Portal, mesenteric</td>
</tr>
<tr>
<td>SM</td>
<td>Woman</td>
<td>80</td>
<td>2000</td>
<td>MF known</td>
<td>None</td>
<td>Portal</td>
</tr>
<tr>
<td>GR</td>
<td>Woman</td>
<td>52</td>
<td>2001</td>
<td>No</td>
<td>None</td>
<td>Portal, mesenteric</td>
</tr>
<tr>
<td>LR</td>
<td>Man</td>
<td>50</td>
<td>2001</td>
<td>MF during follow-up</td>
<td>None</td>
<td>Portal, mesenteric</td>
</tr>
<tr>
<td>TA</td>
<td>Man</td>
<td>42</td>
<td>2002</td>
<td>ET known</td>
<td>None</td>
<td>Portal</td>
</tr>
<tr>
<td>CA</td>
<td>Man</td>
<td>85</td>
<td>2003</td>
<td>No</td>
<td>Hyperhomocysteinemia</td>
<td>Portal</td>
</tr>
<tr>
<td>NC</td>
<td>Man</td>
<td>42</td>
<td>2003</td>
<td>PV known</td>
<td>None</td>
<td>Portal, mesenteric</td>
</tr>
<tr>
<td>MM</td>
<td>Woman</td>
<td>28</td>
<td>2003</td>
<td>No</td>
<td>None</td>
<td>Spleen, portal</td>
</tr>
<tr>
<td>LF</td>
<td>Woman</td>
<td>34</td>
<td>2004</td>
<td>No</td>
<td>Oral contraceptives</td>
<td>Spleen, portal, mesenteric</td>
</tr>
<tr>
<td>DM</td>
<td>Man</td>
<td>80</td>
<td>2006</td>
<td>No</td>
<td>None</td>
<td>Spleen, portal, mesenteric</td>
</tr>
<tr>
<td>NA</td>
<td>Woman</td>
<td>36</td>
<td>2006</td>
<td>MF known</td>
<td>None</td>
<td>Portal, mesenteric</td>
</tr>
<tr>
<td>LA</td>
<td>Woman</td>
<td>38</td>
<td>2006</td>
<td>No</td>
<td>None</td>
<td>Portal</td>
</tr>
</tbody>
</table>

CML, chronic myeloid leukemia; ET, essential thrombocythemia; MF, idiopathic myelofibrosis; PV, polycythemia vera.

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and two ET (two women). Among the 10 patients (three men and seven women) carrying the JAK2 V617F mutation and presenting with PMVT without a diagnosis of MPD, three (one man and two women) were then diagnosed as having MF (n = 2) or PV (n = 1). Thus, in seven patients (two men and five women) a MPD was not detected (Fig. 1). In fact, complete blood counts taken during the follow-up showed hematocrit, hemoglobin, and leukocyte and platelet counts that did not exceed 45%, 145 g L\(^{-1}\), \(8.0 \times 10^9\) L\(^{-1}\), and \(500 \times 10^9\) L\(^{-1}\), respectively. A bone marrow biopsy was carried out in two women (patients DSM and GR) and no sign of hyperplasia or fibrosis was found.

**Discussion**

Splanchnic venous thrombosis is increasingly recognized as a multifactorial disorder, although the specific combination of genetic and acquired risk factors or local precipitating events may differ between patients. Risk factors include genetic, acquired systemic factors and local precipitating factors in the portal/hepatic area. Local factors comprise disorders leading to decreased portal flow, such as liver cirrhosis and hepatobiliary malignancies [26,27]. Of the hereditary factors, deficiencies of the natural anticoagulant proteins, protein C, protein S and antithrombin, FV Leiden and FII G20210A mutation have been reported as risk factors for venous thrombosis both in the presence and the absence of cirrhosis [3,28,29]. In the present study, we confirm that in patients presenting with PMVT but without local precipitating factors, such as liver cirrhosis and hepatobiliary malignancies, inherited thrombophilic risk factors significantly contribute to the multifactorial etiology of venous thrombosis, being present in 21 patients (21.2%).

Systemic, acquired risk factors include uncommon disorders associated with a high relative risk of PMVT (MPD, antiphospholipid syndrome and paroxysmal nocturnal hemoglobinuria), as well as more common factors, such as cancer, inflammation, oral contraceptives/hormone replacement therapy, pregnancy and postpartum, etc. As a whole, a thrombophilic risk factor was identified in about one half of patients (53.5%) presenting with PMVT and the coexistence of causative factors was observed 12 (12.1%), indicating that thrombosis of the portal vein system, like other manifestations of thrombosis, can be a result of combined pathogenetic mechanisms [3,30].

Chronic MPDs, such as PV and ET, follow a chronic clinical course with increased risk of thrombosis and of evolution to MF with myeloid metaplasia or acute leukemia [31,32]. Thrombosis remains the most frequent complication during follow-up and is the main predictor of death in both PV and ET.

We have found a high incidence of the JAK2 V617F mutation in patients with PMVT. Of note, only seven of the 17 JAK2 V617F-positive subjects with PMVT were previously known to have MPDs. Three other JAK2 V617F-positive patients had a diagnosis of MPD after the occurrence of PMVT and therefore represent a group with a ‘latent form’ of MPD. The remaining cases failed to fulfill the accepted diagnostic criteria for MPDs, which suggests that they should be carefully observed for the subsequent development of overt MPDs. MPDs are all associated with clonal hematopoiesis, with the key abnormality originating within the hemopoietic stem cell. Whereas clonality in the majority of cases of chronic myeloid leukemia can be established by Philadelphia chromosome or bcr-abl detection, a clonal cytogenetic abnormality is found in only a minority of cases of ET, PV, and idiopathic MF. Although all but one positive sample were confirmed after the sequencing analysis, none of the patients was homozygous for the JAK2 V617F mutation in circulating blood cells. These
findings are in agreement with previous data in which homozygosity for the JAK2 V617F mutation was detected only in a reduced portion of patients [33]. We have studied total white blood cells. It is conceivable that the analysis of purified neutrophils would have detected patients with a very low percentage of cells carrying the mutant allele [33]. However, present data support the concept that the presence of JAK2 V617F is not sufficient in itself for the MPD phenotype, the subsequent occurrence of which is presumably determined by additional acquired mutations over time in a process of clonal evolution [34].

Results observed in PV have parallels in Chuvash polycythemia, a unique VHL syndrome characterized by homozygous germline mutations of VHL gene, mainly VHL 598C > T homozygotes [17]. In this syndrome, a higher occurrence of splanchic venous thrombosis has been reported [18,19], suggesting that the higher incidence of thrombosis in patients with PV than in those with ET, together with the finding that the JAK2 V617F mutation in ET appears to promote a PV phenotype, may have a common mechanism: a polycythemic condition with an unusually high tendency for thrombosis via increased hematocrit. Although Chuvash polycythemia is a rare condition of erythrocytosis, it is endemic in the southern Italy [35]. Thus, it is conceivable that homozygosity for VHL 598C > T mutation might be identified as risk factor in patients with PMVT. We have found only two heterozygotes and both of them have experienced a PMVT. However, giving the low number of carriers of the mutant allele, present data cannot provide useful insights into this issue.

In conclusion, the JAK2 V617F mutation is frequently found in patients with PMVT and a portion of them do not suffer from an overt MPD. An understanding of the genetic factors predisposing to PMVT is important in identifying subgroup(s) of at-risk patients. Present findings suggest that, in patients presenting with PMVT, screening for the JAK2 V617F mutation may be useful to recognize patients who should be carefully observed for the subsequent development of overt MPDs.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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