Brief report

The JAK2-V617F mutation is frequently present at diagnosis in patients with essential thrombocytopenia and polycythemia vera

Eric Lippert, Marjorie Boissinot, Robert Kralovics, François Girodon, Irène Dobo, Vincent Praloran, Nathalie Boiret-Dupré, Radek C. Skoda, and Sylvie Hermouet

We determined the allelic frequency of the JAK2-V617F mutation in DNA and assessed the expression levels of the mutant and wild-type JAK2 mRNA in granulocytes from 60 patients with essential thrombocytopenia (ET) and 62 patients with polycythemia vera (PV) at the time of diagnosis. Using allele-specific quantitative polymerase chain reaction (qPCR), we detected JAK2-V617F in 75% of ET and 97% of PV at diagnosis. The total JAK2 mRNA levels were elevated in ET, PV, and secondary and idiopathic erythrocytosis, suggesting that hyperactive hematopoiesis alters JAK2 expression. The expression levels of JAK2-V617F mRNA were variable but strongly correlated with the allelic ratio of JAK2-V617F determined in DNA. Thus, differences in JAK2-V617F expression, markedly lower in ET than in PV, reflected different percentages of granulocytes carrying the mutation. Moreover, allelic ratios higher than 50% JAK2-V617F, indicating the presence of granulocytes homozygous for JAK2-V617F, were found in 70% of PV at diagnosis but never in ET. (Blood. 2006;108:1865-1867)

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Introduction

Since the discovery of the JAK2-V617F mutation in BCR-ABL–negative myeloproliferative disorders (MPDs),1-4 several cohorts of patients were studied. Most reports agreed on the presence of JAK2-V617F in a large majority of polycythemia vera (PV), but the rate of positivity in essential thrombocytopenia (ET) varied.5,8 Among the reasons for such differences are ET heterogeneity (patients were often treated), the criteria used for diagnosis, the type of cellular material studied, and the sensitivity of the techniques, allele-specific polymerase chain reaction (AS-PCR) revealing more mutations than sequencing or restriction fragment analysis. This prompted us to develop a sensitive, quantitative AS-PCR (AS-qPCR), which we used to examine patients addressed for diagnosis of polycythemia or thrombocytosis.

Study design

With informed consent and before treatment, blood and bone marrow (BM) samples from 62 PV and 60 ET, as well as 76 controls (24 secondary erythrocytosis [SE], 28 idiopathic erythrocytosis [IE], 24 reactive thrombocytosis), were collected in 5 centers. Blood was also obtained from 40 erythrocytosis [SE], 28 idiopathic erythrocytosis [IE], 24 reactive thrombocythemia [RT], and PV according to Polycythemia Vera Study Group (PVSG) criteria.

We determined the allelic frequency of the JAK2-V617F mutation by using a sensitive, quantitative AS-qPCR (AS-qPCR), which we used to examine patients addressed for diagnosis of polycythemia or thrombocytosis.

The collagen endogenous colony assays and EPO dosage were measured as described previously.9-12 Blood granulocytes were isolated from the lower interface of a Ficoll density gradient, then submitted to erythocyte lysis. RNA or genomic DNA was extracted with Trizol (Invitrogen, Frederick, MD) or QiAmp DNA mini-kit (Qiagen, Valencia, CA); the latter was also used for cells stained with May-Grünwald-Giemsa. The quantitative reverse-transcriptase (RT)–PCR assay of PRV-1 expression was performed as described.13,14

JAK2 AS-qPCRs and RT-qPCRs were performed with specific forward primers (wild-type JAK2 [JAK2-WT]: 5′-GCAGCGGTTTTAGAATTGTGTTTGATG-3′; JAK2-V617F: 5′-GTCAGTGAGATGTTGATG-3′), common reverse primers (cDNA: 5′-GGCGCTCTCTCTTTATTTAGTGCTACGAGA-3′; DNA: 5′-GCAGCGGTTTTAGAATTGTGTTTGATG-3′), and 6-FAM probes (cDNA: 5′-TGGAGACGAGAGTAAGTAAAACTACAGGCT-3′; DNA: 5′-TGGAGACGAGAGTAAGTAAAACTACAGGCT-3′). Copy numbers were determined by comparison with serial dilutions of plasmids obtained by cloning of JAK2 and ABL cDNA or DNA amplicons from U937 (WT) and HEL (V617F) cells into TOPO-TA vectors (Invitrogen).

Results and discussion

Patient characteristics and frequency of JAK2-V617F in ET and PV at diagnosis

Patients with erythrocytosis or thrombocytosis were diagnosed with PV, SE, ET, or RT according to Polycythemia Vera Study Group criteria.


Table 1.

<table>
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<tr>
<th>JAK2-V617F status</th>
<th>PV</th>
<th>ET</th>
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<tbody>
<tr>
<td>All positive</td>
<td>58</td>
<td>26</td>
</tr>
<tr>
<td>All negative</td>
<td>26</td>
<td>12</td>
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In granulocyte cDNA, JAK2-V617F was never found in IE, SE, and RT, or in healthy donors and presurgery patients, but was present in 43 of 60 ET and 59 of 62 PV (Figure 1). In addition, the mutation was found in DNA from May–Grünewald–Giemsa–stained progenitors (1 BM smear and 2 EMCs) for 3 patients considered negative in granulocytes, raising the percentages of JAK2-V617F positivity to 75% (45/60) in ET and 97% (60/62) in PV. The high frequency of JAK2-V617F positivity in our ET series may be explained by the purity of the granulocyte preparations, the fact that diagnosis was established on the presence of EMCs (not a WHO criterion), in addition to BM histology, but mostly by the sensitivity (0.5%) of the qPCR. Indeed, using a different AS-qPCR, Levine et al detected a similar frequency of JAK2-V617F positivity in ET. Of note, the frequency of JAK2-V617F positivity in PV and ET is as high at diagnosis (this series) as after years of disease progression. Hence, detection of JAK2-V617F is an essential diagnostic test for both ET and PV. For the diagnosis of ET, the EMC assay is more sensitive than the detection of JAK2-V617F; it also allows molecular studies of EMCs, more likely to bear the mutation than unselected blood or BM cells.

Expression levels of JAK2-WT and JAK2-V617F mRNA in granulocytes

Relative expression of JAK2-WT and JAK2-V617F was quantitated against plasmidic standard dilutions and normalized for ABL expression. Granulocytes of IE and SE patients expressed significantly higher levels of JAK2-WT (medians: 348 and 452 JAK2-WT/100 ABL, \( P = .001 \) and \( P = .005 \), respectively) than healthy donors and presurgery patients (197 and 174 copies/100 ABL, respectively). When both JAK2-WT and JAK2-V617F were considered, PV and ET granulocytes also expressed significantly more total JAK2 than healthy donors and presurgery patients (median total JAK2/100 ABL: 680 in PV, \( P < .001; 303 \) in ET, \( P = .008 \), suggesting that chronic stimulation of hematopoiesis may upregulate JAK2 expression.

The percentage of total JAK2 represented by JAK2-V617F (%JAK2-V617F) in ET and in PV was then analyzed (Figure 1; Table 1). This percentage can be assimilated to the allelic ratio since %V617F was similar when assessed in cDNA and in genomic DNA from granulocytes of 40 patients (\( r = .909, P < .01; \%\text{V617F in cDNA} = 1.03 \times [\%\text{V617F in genomic DNA}] \)). The percentages of mutant were not close to 50 or 100 as would be the case if all cells were exclusively heterozygous or homozygous for the mutant allele. Rather, they were distributed continuously, as if fractions of granulocytes had different allelic status. Thus, a percentage of mutants more than 50 is necessary and sufficient to affirm homozygosity. In positive PV, JAK2-V617F represented on average 62% of total JAK2 (median: 61%; range: 8%-98%). In all but one positive PV, JAK2-V617F represented more than 25% of total JAK2. Seventy percent of PV expressed more than 50% JAK2-V617F, implying that at least part of the granulocytes was homozygous for the V617F allele. In contrast, JAK2-V617F represented an average of 20% of total JAK2 in ET (\( P < .001 \).
compared with PV), and all but one positive ET expressed less than 40% JAK2-V617F (median: 19%; range: 3%-50%). Thus, the 50% JAK2-V617F threshold revealed more “homozygous” PV at diagnosis than previously reported using sequencing and nonquantitative PCR.1-3 In ET, either the mechanisms leading to JAK2-V617F homozygosity do not exist, or homozygous clones are repressed.

**Effects of JAK2-V617F levels on hematopoietic lineages and disease phenotype**

Percentages of JAK2-V617F correlated with granulocyte expression of PRV-1 (n = 92, r = 0.543, P < .001), confirming PRV-1 expression as a target of JAK2 signaling.1-3 In patients at diagnosis, JAK2-V617F levels correlated only with leucocyte counts (PV: n = 56, r = 0.496, P = .001; ET: n = 42, r = 0.314, P = .043), not with hemoglobin level, hematocrit level, platelet counts, numbers of EECs, or numbers of EMCs. Consistently, the level of mutant expression was not sufficient per se to determine PV or ET phenotype: comparison of PV and ET with similar levels of mutant (25%-40%) showed similar leucocyte counts, but PV patients had significantly higher EPO, EEC formation, red cell mass (152% vs 78%, P = .001), hematocrit, and hemoglobin level and lower platelet counts (Table 1). However, as recently described for ET,8 in our series of PV and ET at diagnosis, JAK2-V617F was associated with stimulation of erythropoiesis and repression of thrombopoiesis: PV with 75% or more mutant and positive ET differed from other PV and negative ET by higher hematocrit and hemoglobin levels, but lower platelet counts (Table 1).

In summary, sensitive qPCRs detected JAK2-V617F in 97% of PV and 75% of ET at diagnosis, with higher levels of expression in PV than in ET; cells homozygous for the mutation were present in 70% of PV. This demonstrates the interest of precise and sensitive assessment of JAK2-V617F for the diagnosis of MPD.

**Acknowledgments**

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**References**

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