JAK2 V617F prevalence in Brazilian patients with polycythemia vera, idiopathic myelofibrosis and essential thrombocythemia

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Abstract

Polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF) are myeloproliferative disorders (MPD) that arise from the clonal proliferation of a pluripotent hematopoietic progenitor, leading to the over-production of one or more myeloid lineages. Recently, a specific mutation in the JAK2 gene, which encodes a tyrosine kinase, has been shown to be associated with the myeloproliferative phenotype observed in PV, ET and IMF. In this study of Brazilian patients, the JAK2 V617F mutation [c.1887G > T] was detected in four out of 49 patients with PV (96%), 14 out of 25 patients with IMF (56%), and in eight out of 29 patients with ET, which is in accordance with previous screenings of this mutation in other populations.

Key words: JAK2 V617F, myeloproliferative disorders, polycythemia vera, idiopathic myelofibrosis, essential thrombocythemia.

Received: July 14, 2006; Accepted: November 26, 2006.
Peripheral blood samples from eight healthy volunteers were used as controls, since no healthy individuals have been shown to harbor the JAK2 V617F mutation (Baxter et al., 2005; James et al., 2005; Kralovics et al., 2005; Levine et al., 2005; Zhao et al., 2005). The study protocol was approved by the local ethics committee, and informed consent was obtained from all patients. Genomic DNA was extracted using the GFX Genomic Blood DNA Purification Kit (Amersham-Life Science). The presence of the JAK2 V617F mutation was assessed as previously described (Baxter et al., 2005). JAK2 amplicons were obtained using primers JAK2 forward (5'-GGGTTCCTCAGAACGTT GA-3') and JAK2 reverse (5'-TCATTGCTTTCCTTTT CACAA-3'). PCR amplifications were performed in 50 μL reaction mixes containing 50-100 ng of genomic DNA, 0.2 mM dNTP’s, 2 mM MgCl2, 0.2 pmol of each primer, 1X PCR buffer, and 1U Taq DNA polymerase. The cycling parameters were as follows: 96 °C for 2 min followed by 45 cycles at 96 °C for 30 s, 57 °C for 30 s, and 72 °C for 1 min. The 460 bp PCR product (Figure 1A) was submitted to Bsa XI (New England Biolabs Inc.) digestion, for 16 h at 37 °C, and analyzed on a 2% agarose gel. The JAK2 wild-type allele yields 241 bp, 189 bp and 30 bp fragments, while the JAK2 V617F allele remains undigested, since the mutation causes loss of the enzyme site. The Bsa XI digestion pattern in JAK2 V617F-negative patients and healthy controls is shown in Figure 1B, lanes 1 and 4. Patients who are positive for the mutation have both the undigested 460 bp fragment, corresponding to the JAK2 V617F allele, and the Bsa XI fragments of the JAK2 wild-type allele (Figure 1B, lanes 2 and 3). The intensity of the digested fragments visualized on the agarose gel varies from patient to patient, due to differences in the homozygous or heterozygous status of the mutation and in the proportion of clonal cells in the total population. This appears to be a time- and cost-effective methodology for the detection of the JAK2 V617F mutation.

Among the 49 patients with PV studied, the JAK2 V617F mutation was detected in 47 (96%). Among the patients with IMF, 14 out of 25 (56%) had the mutation, while eight (28%) of the 29 ET patients were positive. These frequencies are in agreement with those previously reported (Table 1).

There are already studies in the literature on the contribution of the JAK2 mutation to clinical status and disease severity. A prospective study suggests that two ET subtypes can be defined according to the JAK2 genotype, and that ET patients carrying the JAK2 V617F mutation have phenotypic similarities with PV patients (Baxter et al., 2005). A multicentric study of IMF patients demonstrated that there is no correlation between many of the clinical features and the presence of the JAK2 V617F mutation, but, interestingly, JAK2 V617F-positive patients had a decreased survival (Campbell et al., 2006). It has been shown that, while most of the clinical characteristics of PV did not differ between JAK2 V617F homozygous and heterozygous patients, the former had higher hemoglobin levels at the time of diagnosis, increased incidence of pruritus, higher rates of fibrotic transformation, and higher PRV-1 transcript levels in granulocytes (Tefferi et al., 2006).

The discovery of this mutation has direct implications in the establishment of diagnosis protocols and in the management of patients (Campbell and Green, 2005), and has a great potential for the classification of MPDs and for the development of target therapy (Vainchenker and

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**Table 1** - Frequencies of the JAK2 V617F mutation in Brazilian patients and in patients from the literature presenting with polycythemia vera, idiopathic myelofibrosis and essential thrombocythemia.

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<td>Polycythemia vera</td>
<td>47/49 (96%)</td>
<td>71/73 (97%)</td>
<td>40/45 (89%)</td>
<td>83/128 (65%)</td>
<td>121/164 (74%)</td>
<td>20/24 (84%)</td>
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<td>Idiopathic myelofibrosis</td>
<td>14/25 (56%)</td>
<td>8/16 (50%)</td>
<td>3/7 (43%)</td>
<td>13/23 (57%)</td>
<td>16/46 (35%)</td>
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<td>Essential thrombocythemia</td>
<td>8/29 (28%)</td>
<td>29/51 (57%)</td>
<td>9/21 (43%)</td>
<td>21/93 (23%)</td>
<td>37/115 (33%)</td>
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JAK2 genotyping of patients with PV, IMF and ET is likely to become a clinically useful assay, and sensitive, effective techniques for the routine detection of JAK2 V617F are being developed and tested (James et al., 2006; McClure et al., 2006). Among the questions to be addressed in the investigation of the molecular pathogenesis of MPDs is how a single mutation can give rise to three phenotypically different diseases.

Acknowledgments

This study was supported by grants from FAPESP and CAPES.

References


Associate Editor: Peter L. Pearson