Genetics and Molecular Biology, 30, 2, 336-338 (2007)

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Short Communication

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 overproduction 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.

Polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (IMF) and chronic myelocytic leukemia (CML) are myeloproliferative disorders (MPD) that have been shown to arise clonally from a pluripotent hematopoietic stem cell (Fialkow *et al.*, 1967; Adamson *et al.*, 1976; Jacobson *et al.*, 1978; Fialkow *et al.*, 1981). Important manifestations of these disorders are bone marrow hypercellularity and pan-myeloid myeloproliferation, leading to overproduction of one or more hematopoietic lineages (Spivak *et al.*, 2003). In addition, constant hallmarks of MPD bone marrow cells are their hypersensitivity to several cytokines (Prchal and Axelrad, 1974; Dai *et al.*, 1992; Correa *et al.*, 1994; Dai *et al.*, 1994) and their ability to generate EPO-independent erythroid colonies *in vitro* (Prchal and Axelrad, 1974), commonly referred to as endogenous erythroid colonies (EECs). Recently, gene *JAK2*, which encodes a tyrosine kinase required for effective signaling in response to several cytokines (Parganas *et al.* 1998), was found mutated in these conditions. A somatic mutation, c.1887G > T (p.Val617Phe), ref. sequence NM_004972, commonly known as *JAK2* V617F, has been described in the majority of patients with PV and in a subset of patients with ET and IMF (Baxter *et al.*, 2005; James *et al.*, 2005; Kralovics *et al.*, 2005; Levine *et al.*, 2005; Zhao *et al.*, 2005). It is noteworthy that among the growth factors to which MPD hematopoeitic progenitors are hypersensitive, EPO, SCF, GM-CSF, IL3, TPO and IGF-1 use JAK2 for signaling (Kaushansky, 2005). It has been demonstrated that the valine-to-phenylalanine substitution at amino acid position 617 leads to constitutive tyrosine phosphorylation activity and promotes cytokine hypersensitivity (James *et al.*, 2005; Kralovics *et al.*, 2005; Levine *et al.*, 2005; Zhao *et al.*, 2005). Moreover, EEC colonies cloned from PV patients were all shown to carry the mutation (Baxter *et al.*, 2005), and lethally-irradiated mice transplanted with *JAK2* V617F-expressing bone marrow developed substantial erythrocytosis (James *et al.* , 2005). These observations indicate that this mutation contributes to the myeloproliferative phenotype, a finding with direct implications in the establishment of diagnosis protocols and the patient management (Campbell and Green, 2005). Establishing the prevalence of this mutation in patients with PV, ET and IMF is of practical importance. This prompted us to carry out what is, to our knowledge, the first screening of the *JAK2* V617F mutation in Brazilian patients with PV, ET and IMF.

Peripheral blood samples were obtained from 49 patients with PV, 25 patients with IMF, and 29 patients with ET, seen at the Haematology and Haemotherapy Centre (UNICAMP, Brazil), from July through December, 2005. The diagnoses of PV, IMF and ET were made according to the World Health Organization (WHO) criteria, based on peripheral blood counts and bone marrow histology. Pe-

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ripheral 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'-GGGTTTCCTCAGAACGTT GA-3') and *JAK2* reverse (5'-TCATTGCTTTCCTTTTT 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 $MgCl_2$, 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 *Bsa* XI 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

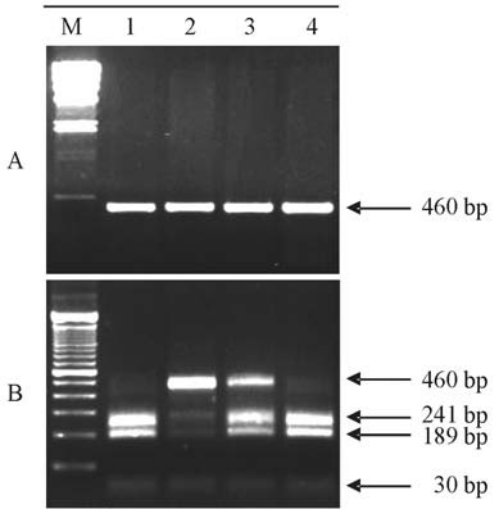

Figure 1 - *JAK2* V617F screening by PCR and *Bsa* XI digestion. Lanes 1-3: Patients with (1) essential thrombocythemia, ET; (2) polycythemia vera, PV; (3) idiopathic myelofibrosis, IMF. Lane 4: Control individual. **(A)** 460 bp undigested PCR product; lane M, λ-*Hind* III fragments (New England BioLabs Inc.); **(B)** *Bsa* XI digestion of the 460 bp PCR product yields 241 bp, 189 bp and 30 bp fragments from the wild-type allele; the fragment corresponding to the *JAK2* V617F mutation remains undigested: normal alleles in a patient with ET (1); a normal and a mutated allele in patients with PV (2) and IMF (3); normal alleles in a control individual (4). Lane M, 100 bp molecular weight ladder (New England BioLabs Inc.).

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 (Campbell *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

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.

Myeloproliferative disorders	This study	Baxter *et al.* (2005)	James *et al.* (2005)	Kralovics *et al.* (2005)	Levine *et al.* (2005)	Zhao *et al.* (2005)
Polycythemia vera	47/49 (96%)	71/73 (97%)	40/45 (89%)	83/128 (65%)	121/164 (74%)	20/24 (84%)
Idiopathic myelofibrosis	14/25 (56%)	8/16 (50%)	3/7 (43%)	13/23 (57%)	16/46 (35%)	-
Essential thrombocythemia	8/29 (28%)	29/51 (57%)	9/21 (43%)	21/93 (23%)	37/115 (33%)	-

Constantinescu, 2005). *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.

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Associate Editor: Peter L. Pearson