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JAK2 V617F Mutation Prevalence in Myeloproliferative Neoplasms in Pernambuco, Brazil

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Background: The *JAK2 V617F* mutation is associated with three myeloproliferative neoplasms (MPNs): polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). It generates an unregulated clonal hematopoietic progenitor and leads to abnormal increased proliferation of one or more myeloid lineages. Subjects bearing this mutation may present more frequently with complications such as thrombosis and bleeding, and no specific treatment has yet been developed for *BCR-ABL*-negative *JAK2 V617F*-negative MPNs. ***Aims:*** To determine the prevalence of *JAK2 V617F* in MPNs in Pernambuco, Brazil, and to compare it with previous studies. *Material and Methods:* 144 blood samples were collected at the Hospital of Hematology of the HEMOPE Foundation and were genotyped by polymerase chain reaction–restriction fragment length polymorphism with BsaXI enzymatic digestion. *Results and Discussion:* 88% (46/52) of the patients with PV, 47% (39/81) with ET, and 77% (8/11) with PMF were positive for *JAK2 V617F*, while more than 35% of the individuals were *JAK2 V617F*-negative, confirming a high prevalence of this abnormality in MPNs, more frequently with a low mutated allele burden, similar to what has been reported in other Western countries, despite differences among methods used to detect this mutation. Screening for *JAK2 V617F* may allow specific management of these diseases with JAK2 inhibitors in the future and highlights the need for further studies on the pathogenesis of *BCR-ABL*-negative *JAK2 V617F*-negative MPNs.

Introduction

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic diseases in which one or more myeloid lineages present with abnormally increased levels of cell proliferation. They are characterized by a multipotent progenitor cell that proliferates independently from physiological levels of growth factors such as erythropoietin (EPO) and thrombopoetin, leading to accelerated hematopoiesis and bone marrow hypercellularity (Baxter *et al.*, 2005). Differently from chronic myeloid leukemia, *BCR-ABL*-negative MPNs have variable association with bleeding, thrombotic events, marrow fibrosis, and less frequently blastic transformation (Lussana *et al.*, 2009).

JAK2 is a member of the janus kinases (JAKs) family, a group of cytoplasmic tyrosine kinases that transduce cytokine-mediated signals via the JAK-signal transducers and activators of transcription (STAT) pathway. Four JAKs have been described: JAK1, JAK2, JAK3, and TYK2 (Ward *et al.*, 2000). When activation of a receptor by a cytokine occurs, JAK2 becomes phosphorylated and causes further phosphorylation and dimerization of the STAT5 protein. STAT5 dimers enter the nucleus and act as transcription factors for gene regulation. The *JAK2* gene is localized in chromosomal region 9p24 and was first cloned in 1989 in a cDNA library of a murine cell lineage (Wilks, 1989).

The *JAK2 V617F* point mutation generates the substitution of a guanidine for a thymidine in the encoding sequence (1848G > T), resulting in the exchange of a valine residue for a phenylalanine at position 617 of the mutated JAK2 protein. This appears to relieve the inhibitory effect of the pseudokinase domain (JH2) on the kinase activity domain (JH1), leading to an unregulated proliferation in myeloid/erythroid cells (Baxter *et al.*, 2005; James *et al.*, 2005; Vainchenker *et al.*, 2011). JAK2 constitutive activation causes hypersensitivity to several cytokines known to stimulate hematopoietic

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precursors, such as EPO, thrombopoietin, interleukin 3, granulocyte-macrophage colony-stimulating factor, and stem cell factor (Levine and Wernig, 2006; Monte-Mór *et al.*, 2007). Other changes in mutated cells involve STAT5-mediated signaling, extracellular signal regulated kinases, and phosphoinositol kinase/Akt pathways (Parganas *et al.*, 1998; Campbell and Green, 2006; Lippert *et al.*, 2006).

This mutation is now recognized as an important oncogenic event responsible for the development of *BCR-ABL*-negative MPNs, such as polycythemia vera (PV) rubra, essential thrombocythemia (ET), and primary myelofibrosis (PMF). The discovery of the *JAK2 V617F* mutation was most important to the diagnosis of PV, as it is present in more than 90% of individuals with this MPN. This allowed better characterization of PV in contrast to other MPNs and validated the clinical diagnostic criteria for this disease. (Tefferi *et al.*, 2007).

JAK2 V617F has been found in more than 90% of PV cases, but also in up to 60% of ET and PMF patients (Monte-Mór *et al.*, 2007; Haferlach *et al.*, 2008; Scott *et al.*, 2009). Since the mutation is not universally present, other genetic events are believed to be involved in the etiopathogenesis of both ET and PMF, as well as *JAK2 V617F*-negative PV.

Several research groups have reported the somatic mutation V617F in the gene encoding JAK2 in patients with *BCR-ABL*-negative MPNs, and molecular studies in different regions of the world, such as the United States, Europe, Middle East, India, Korea, Japan, Argentina, and Southeastern Brazil, have investigated its prevalence (Baxter *et al.*, 2005; James *et al.*, 2005; Kravolics *et al.*, 2005; Levine and Wernig, 2006; Monte-Mór *et al.*, 2007; Bang *et al.*, 2009; Basquiera *et al.*, 2009; Sazawal *et al.*, 2010; Ayad and Nafea, 2011; Mahfouz *et al.*, 2011).

The aims of this study were to determine the prevalence of *JAK2 V617F* in patients with *BCR-ABL*-negative MPNs at the Hospital of Hematology at the HEMOPE Foundation, and to compare our results with previous studies.

Materials and Methods

This study was performed after approval by the local ethics committee of the HEMOPE Foundation. All samples were collected on informed consent.

Patients were recruited at the Hospital of Hematology in Recife, Pernambuco, Brazil, based on the clinical diagnosis of the main three *BCR-ABL*-negative MPNs (PV, ET and PMF). Between May 2009 and August 2010, 144 peripheral blood samples were collected in vacuum collecting tubes containing ethylenediaminetetraacetic acid as anticoagulant.

Peripheral blood granulocytes were separated by gradient centrifugation with Ficoll-Paque (Sigma) to enhance test sensitivity (Hermouet *et al.*, 2007). DNA was extracted using standard phenol-chlorophorm protocol (Davis *et al.*, 1986). We performed polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) with DNA amplification by specific primers (forward primer: 5′ - GGG TTT CCT CAG AAC GAA CGT TGA - 3′; reverse primer: 5′ - TCA TTG CTT TCC TTT TTC ACA A - 3′), yielding a 460 base pair (bp)-long fragment. This reaction was followed by digestion with *Bsa*XI enzyme (New England BioLabs Inc.), and samples were analyzed by electrophoresis in 3% agarose gel. Genotypes were confirmed in 24 samples with suboptimal PCR amplification by direct DNA sequencing on a MegaBACE 1000 DNA Analysis System (GE Healthcare).

In normal subjects, the restriction enzyme *Bsa*XI acts on specific binding sites by cutting the PCR product into three fragments: 241, 189, and 30 base pairs long. Fragments bearing the V617F mutation have their restriction site abolished. Thus, samples with mixed cellularity due to a relatively lower mutation burden present with four fragments (460, 241, 189, and 30 bp), while samples with a high mutated allele burden present with only 460 bp-long, digestion-resistant fragments.

Results and Discussion

A total of 144 samples were collected: 52 PV (18 male, mean age 69 ± 11 years), 81 ET (23 male, mean age 63 ± 18 years), and 11 PMF (5 male, mean age 63 ± 10 years). One study had suggested that sex could be a determining factor, as ET was more common in women, while men were predominant in PV (Campbell and Green, 2006). In the present study, women were more frequently found in both PV (65.4%) and ET (71.6%) groups.

Among the PV samples, *JAK2 V617F* was detected in 46 (88%). Thirty-nine of 81 (47%) ET patients bore the mutation, while 8 (77%) of 11 PMF patients were *JAK2 V617*-positive. Samples screened positive for *JAK2 V617F* were further classified according to the pattern of fragments obtained in the PCR-RFLP reactions as "high burden" or "low burden." These data are summarized in Table 1.

Our data show that, when present in PV and ET patients, the mutated *JAK2 V617F* allele burden was more frequently low (63% and 74%, respectively). Higher mutation burden rates were found in both diseases compared with previous reports (25%–30% in PV and 2% in ET) (Baxter *et al.*, 2005; Kralovics *et al.*, 2005; Levine *et al.*, 2005), although still lower than rates found in an Indian population (Sazawal *et al.*, 2010). In the latter study, the mutation was also determined by PCR-RFLP, so a possible explanation for a higher mutation burden in both India and Brazil could be that patients present later in the course of the diseases in these developing countries, as suggested by Sazawal and collaborators.

Table 2 compares the findings of this study with previously published work and highlights that different methods are available to detect *JAK2 V617F*, for example, allele-specific PCR (AS-PCR), real-time PCR (RT-PCR), multiple direct DNA sequencing methods, including mass spectrometry-based platforms and, as has been the main approach of the current study, PCR-RFLP. Sensitivity among the methods

Table 1. Mutated Allele Burden for *JAK2 V617F* in Patients with *BCR/ABL*-Negative Myeloproliferative Neoplasms Followed at the HEMOPE Foundation

Diagnosis	*JAK2 V617F high burden*	*JAK2 V617F low burden*	*JAK2 V617F negative*	*Total*
PV	17 (32%)	29 (56%)	6 (12%)	52
ET	10 (12%)	29 (35%)	42 (53%)	81
PMF	7 (63%)	1 (14%)	3 (23%)	11

Data are represented as absolute number (percentage of patients in each group).

PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis.

Table 2. Comparison of the Prevalence and Methods Used for Detection of *JAK2 V617F* in *BCR/ABL*-Negative Myeloproliferative Neoplasms Among Different Studies

Study (number of patients with PV, ET or PMF)	*Methods*	*Polycythemia vera*	*Essential thrombocythemia*	*Primary myelofibrosis*
Silva *et al.* (present study)		88% (46)	47% (39)	77% (8)
($n=144$)	PCR-RFLP	$n=52$	$n=81$	$n=11$
Mahfouz *et al.* (2011)		100% (13)	68% (28)	NR
($n=54$)	RT-PCR	$n=13$	$n=41$	NR
Ayad and Nafea (2011)		81% (44)	57% (17)	46% (18)
($n=123$)	AS-PCR	$n=54$	$n=30$	$n=39$
Tognon *et al.* (2011)		83% (10)	NR	NR
($n=49$)	RT-PCR	$n=12$	$n=26$	$n=11$
Sazawal *et al.* (2010)		82% (28)	70% (7)	52% (16)
($n=75$)	PCR-RFLP	$n=34$	$n=10$	$n=31$
Zhang *et al.* (2010)		82% (73)	36% (52)	51% (24)
($n=278$)	AS-PCR, DNA seq, MS	$n=89$	$n=142$	$n=47$
Basquiera *et al.* (2009)		89% (40)	69% (30)	47% (7)
($n=103$)	AS-PCR	$n=45$	$n=43$	$n=15$
Monte-Mór *et al.* (2007)		96% (47)	28% (8)	56% (14)
($n=103$)	PCR-RFLP	$n=49$	$n=29$	$n=25$
Levine *et al.* (2005)		74% (121)	33% (37)	35% (16)
($n=325$)	DNA seq, MS	$n=164$	$n=115$	$n=46$
Baxter *et al.* (2005)		97% (71)	57% (29)	50% (8)
($n=140$)	AS-PCR, DNA seq	$n=73$	$n=51$	$n=16$
Kravolics *et al.* (2005)		65% (83)	23% (21)	57 (13)
($n=244$)	DNA seq	$n=128$	$n=93$	$n=23$
James *et al.* (2005)		89% (40)	43% (9)	43% (3)
($n=73$)	DNA seq	$n=45$	$n=21$	$n=7$

Data are represented as percentage of patients in each group (absolute number).

NR, not reported; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; RT-PCR, real-time polymerase chain reaction; AS-PCR, allele-specific polymerase chain reaction; DNA seq, DNA sequencing; MS, mass spectrometry-based method.

varies and has been well reviewed elsewhere (Steensma, 2006; Cankovic *et al.*, 2009). Briefly, PCR-RFLP has been demonstrated to be able to detect down to 20% of mutated DNA on a wild-type background (Baxter *et al.*, 2005; James *et al.*, 2005), and automated sequencing using autoradiography as commonly used in the past was even less sensitive. Current DNA sequencing methods may prove sensitive down to 5%–10% of mutated DNA depending on the sample type (cell lines or human) and purity, but RT-PCR techniques have increased sensitivity to 2%–4%, and AS-PCR-based studies have reported down to 0.01% of detectable *JAK2 V617F*-positive DNA (McClure *et al.*, 2006). Nevertheless, variation in mutation prevalence in the compared studies considered appears to rely more on sample number disparity than on differences among methods used, further confirming the utility of PCR-RFLP as a simple and cost-effective method to screen this mutation.

Despite a multicentric study having demonstrated that the presence of *JAK2 V617F* in PMF does not determine any specific clinical manifestation, such patients present with poorer survival (Campbell *et al.*, 2006). We found that 77% of the PMF patients were JAK2 V617-positive. Even though our small number of patients ($n=11$) does not allow to draw any definitive conclusion about the mutation prevalence in this disease in our population, this finding still calls attention to the need for target-specific therapies for this kind of MPN with a worse outcome.

The prevalence of the *JAK2 V617F* mutation in MPNs in this study was similar to what has been described by other groups around the globe, and confirms that its high prevalence among PV patients allows a more confident diagnosis of this disease. Nonetheless, the diagnosis of all BCR-ABL-negative MPNs, particularly *JAK2 V617F*-negative PV, should rely on the criteria published in the 2008 WHO classification (Tefferi *et al.*, 2007). A negative screening for *JAK2 V617F*, found in over a third (51/144) of the patients in this study, should not be considered evidence enough to discard the diagnosis of a MPN. Still, screening for this mutation may help physicians narrow down their diagnostic possibilities when a *BCR-ABL*-negative MPN is suspected, as positivity for *JAK2 V617F* is virtually 100% specific for the distinction between PV and other causes of increased hematocrit (James *et al.*, 2006).

Previous studies in Brazil have been performed only in the Southeastern part of the country, in the State of São Paulo (Monte-Mór *et al.*, 2007; Tognon *et al.*, 2011). This is the first study in the Brazilian Northeastern population, and while our data did not differ from other Brazilian or worldwide populations, this suggests that the development of *JAK2 V617F*-positive MPNs may occur rather independently from an ethnical background. The availability of *JAK2 V617F* screening may help define better therapeutic strategies for patients with MPNs in our region in the future, especially considering current promising developments in clinical trials with JAK2 inhibitors (Verstovsek *et al.*, 2010; Pardanani *et al.*, 2011). Moreover, this study highlights the need for collaboration in further molecular studies on the pathogenesis of *BCR-ABL*-negative *JAK2 V617F*-negative MPNs.

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Author Disclosure Statement

No competing financial interests exist.

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