



## ARTÍCULO ORIGINAL

# The Janus Kinase 2 (JAK2) V617F mutation in hematological malignancies in México

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## ABSTRACT

A new mutation (V617F) affecting the *JAK2* gene has been recently described as acquired in patients with myeloproliferative disorders and other myeloid malignancies. Using an amplification refractory mutation system, we investigated this mutation in 70 Mexican mestizo patients with hematological malignancies: 28 cases of acute lymphoblastic leukemia, 17 cases of Ph1-positive chronic myelogenous leukemia, 8 patients with acute myelogenous leukemia, 6 patients with chronic lymphocytic leukemia, 6 patients with polycythemia vera (PV), two patients with essential thrombocythemia (ET), one patient with hypereosinophilic syndrome one patient with primary myelofibrosis (MF) and one patient with chronic myelomonocytic leukemia. The mutation was identified in 4 of 6 patients with PV, in one of 2 patients with ET and in the patient with MF. Our data add to the observation that the JAK2 V617F mutation seems to be rather uncommon in myeloid malignancies other than the classic BCR/ABL negative MPD.

**Key words.** JAK2 V617F mutation. Myeloproliferative. Disorders. México.

## INTRODUCTION

Janus (*ianua*), the Roman god of gates and doors, of beginnings and endings, is represented with a double-faced head, each looking in opposite directions; he is also called *bifrons* (two fronts or two faces). Protein kinases (PK) are enzymes that catalyze protein phosphorylation, whereas protein phosphatases

## La mutación V617F del gen de la cinasa JAK2 en padecimientos hematológicos malignos en México

### RESUMEN

Se ha descrito una nueva mutación (V617F) que afecta al gen de la cinasa JAK2 en pacientes con padecimientos mieloproliferativos y otras neoplasias mieloides. Empleando un sistema de amplificación de mutaciones refractarias y reacción en cadena de la polimerasa, investigamos esta mutación en 70 pacientes mestizos mexicanos con neoplasias hematológicas malignas: 28 casos de leucemia aguda linfoblástica, 17 casos de leucemia granulocítica crónica BCR/ABL (+), ocho casos de leucemia aguda mieloblástica, seis casos de leucemia linfocítica crónica, seis casos de policitemia vera (PV), dos casos de trombocitosis primaria (TP), un caso de síndrome hipereosinofílico primario y un caso de mielofibrosis primaria (MF) y un caso de leucemia mielomonocítica crónica. La mutación se identificó en cuatro de seis pacientes con PV, en uno de dos pacientes con TP y en el paciente con MF. Estos datos confirman que esta mutación es infrecuente en neoplasias hematológicas mieloides diferentes a los síndromes mieloproliferativos malignos negativos al BCR/ABL; es probable que esta mutación se convierta en el marcador molecular de la PV.

**Palabras clave.** Mutación JKZ V617F. Síndromes mieloproliferativos. México.

do the opposite: regulate PK activity through protein dephosphorylation. Protein-tyrosine kinases (PTK) are PK that catalyze the transfer of the  $\gamma$ -phosphate group of adenosine triphosphate (ATP) to the hydroxyl groups of specific tyrosine residues in signal transduction molecules. In humans, the Janus PTK family (JAKs), has two similar domains facing in opposite directions –hence its name of roman origin–

and contains four members: JAK1, JAK2, JAK3 and TYK2. JAKs phosphorylate signal transducers and activators of transcription (STATs) simultaneously with other phosphorylations required for activation.<sup>1</sup>

Nowell and Hungerford<sup>2</sup> described in 1960 the first disease-specific cytogenetic marker, the Philadelphia chromosome (Ph1) in chronic myelogenous leukemia (CML). Since then, other molecular markers of leukemia and myeloproliferative disorders (MPDs) have been described.<sup>3</sup> A new mutation (V617F) affecting the *JAK2* gene has been recently described as acquired in a large proportion of patients with MPDs and other myeloid disorders.<sup>3,4</sup> We report herein the results of looking for the *JAK2* V617F mutation in a group of 70 Mexican mestizo patients with different hematologic malignancies.

## MATERIAL AND METHODS

### Patients

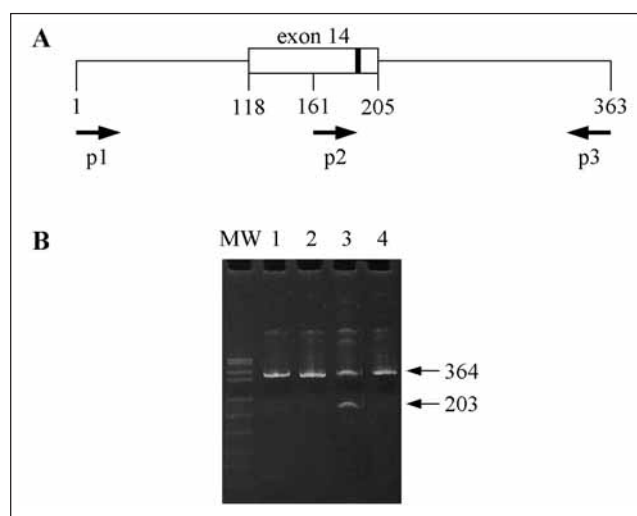
Patients with hematological malignancies studied at Centro de Hematología y Medicina Interna de Puebla after March 2005 were prospectively accrued in the study; in addition, DNA samples from our bank were also studied. The diagnosis and classification of leukemia were done according to conventional criteria;<sup>5</sup> patients were studied, treated and followed by one of us. As normal controls 150 healthy blood donors were studied. The operational definition of mestizo, recently published by Pons-Estel *et al.*<sup>6</sup> was employed to select the patients: Individuals born in Latin America who had both Amerindian and white ancestors.

### Analysis of the *JAK2* V617F mutation

An amplification refractory mutation system (ARMS) method was used according to Baxter *et al.*<sup>7</sup> Briefly, genomic DNA was isolated from peripheral blood leukocytes according to standard procedures. In a multiplex format, the mutation was detected with the help of allele specific primers (203 bp) and the complete exon 12 was amplified as an internal amplification control (364 bp), taking care to not exceed 0.05 µg of DNA per 50 µl amplification reaction. Amplification products were analyzed after electrophoresis on 4.5% polyacrylamide gels (Figure 1).

## RESULTS

Of the 70 samples with a known diagnosis of a hematological malignancy, there were six cases posi-



**Figure 1.** Detection of *JAK2* V617F mutation. Panel A shows the genomic situation of exon 14 (empty rectangle), codon 617 (black rectangle) and the position of the primers used for the detection (arrows). Primer pair p1/p3 is used as internal amplification control, primer pair p2/p3 for the specific amplification of the mutated allele. In B an electrophoretic analysis is shown. Patients 1, 2, and 4 are negative for the V617F mutation, patient 3 is positive (presence of the 203 bp amplification product). MW: Molecular weight marker. Sizes and positions are indicated in bp.

**Table 1.** Results of searching for the *JAK2* V617F mutation in Mexican mestizo patients with different hematological malignancies. N: number; Ph1: Philadelphia chromosome.

Condition	n	JAK V617F mutation
Ph1 (-) polycythemia vera	6	4
Ph1 (-) hypereosinophilic syndrome	1	0
Ph1 (+) chronic myelogenous leukemia	17	0
Ph1 (-) primary thrombocytemia	2	1
Ph1 (-) primary myelofibrosis	1	1
Acute myelogenous leukemia	8	0
Acute lymphoblastic leukemia	28	0
B cell chronic lymphocytic leukemia	6	0

Chronic myelomonocytic leukemia.

tive for the *JAK2* V617F mutation. The table 1 shows these results.

The patient with primary myelofibrosis was allografted from his HLA identical sibling; interestingly, the detection of the V617F affecting the *JAK2* gene has diminished substantially as the patient becomes a chimera.

## DISCUSSION

Chronic myeloproliferative diseases are clonal hematopoietic stem cell disorders characterized by pro-

liferation of one or more myeloid cell lineages in the bone marrow and increased numbers of mature and immature cells in the peripheral blood.<sup>3,8-9</sup> CMPDs include polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (MF) and CML, plus rarer subtypes such as chronic neutrophilic leukemia, hypereosinophilic syndrome, and chronic eosinophilic leukemia. These diseases overlap with myelodysplastic/myeloproliferative diseases such as atypical CML and chronic myelomonocytic leukemia, in which proliferation is accompanied by dysplastic features or ineffective hematopoiesis in other lineages.<sup>3,10</sup> Although there are stringent diagnostic criteria for CMPDs subtypes, precise categorization remains a subject of debate and furthermore, it can be difficult to differentiate some cases from reactive disorders. Only CML is characterized by a pathognomonic molecular marker, the *BCR-ABL* fusion, and the primary abnormalities driving excess proliferation in most other cases have been obscure. Several lines of evidence have implicated aberrant PTK signaling as the root cause of some CMPDs. *BCR-ABL* itself is a constitutively active tyrosine kinase that is believed to be the primary, and probably the only, driving force behind chronic-phase CML.<sup>3,10</sup> Other gene fusions have been identified in rare cases of CMPD's that involve the tyrosine kinases PDGFRA, PDGFRB, FGFR1, and JAK2.<sup>3,10</sup> In addition, the KIT receptor is activated by a point mutation in the majority of cases of systemic mastocytosis. To investigate the molecular pathogenesis of CMPDs, mutation screen studies for genes encoding tyrosine kinases and down stream signaling components have been conducted.<sup>3,10</sup>

The molecular markers of several hematological malignancies may have a different distribution worldwide. We have shown that in México, the prevalence of the bcr1 subtype of the PML/RAR $\alpha$  fusion gene is significantly higher than that informed in Caucasians and similar to that in Asians,<sup>11</sup> whereas the distribution of the bcr subtypes of the *BCR/ABL* fusion gene is similar to that described in Caucasian.<sup>12</sup> These "molecular" differences may account for some of the differences in the prevalence of several hematologic malignancies in certain populations, such as the high prevalence of the promyelocytic leukemia in México.<sup>13</sup>

CMPDs are less frequent in México than in Caucasian populations;<sup>9</sup> of the CMPDs, PV is the less frequent one in Mexican mestizos.<sup>9</sup> In this study, we have found a similar prevalence of the *JAK2* V617F mutation in patients with different hematological malignancies. The *JAK2* V617F mutation could

eventually become the molecular marker of PV, but it also occurs in other myeloproliferative disorders;<sup>3,4,10</sup> we found the mutation in 4 of 6 patients with PV, in one of 2 patients with ET and in the patient with MF, while all patients positive for the Philadelphia chromosome were negative as published previously.<sup>10,14</sup> Our data add to the observation that the *JAK2* V617F mutation seems to be rather uncommon in myeloid malignancies other than the classic *BCR/ABL* negative MPD and that the V617F-negative MPD are likely to reflect mutations in other molecules that modulate the JAK/STAT pathway, or mutations in different signaling pathways.<sup>14</sup> Despite the fact that the *JAK2* V617F is not present in all cases of PV,<sup>15</sup> it is possible that it will eventually become the molecular marker of this disease;<sup>3,4,14-16</sup> on the other hand, and at the very least, this newly identified molecular lesion will most likely form the basis of a new classification of the CMPDs<sup>15</sup> and of the definition of the "molecular remission" of these diseases.<sup>17</sup>

The identification of molecular markers of diseases may have therapeutic implications. It is a tantalizing prospect that one might be able to modulate selected JAK/STAT-mediated cellular signals by inhibiting JAK kinase activity in order to achieve a positive therapeutic outcome; while current data suggest no therapeutic use for JAK1 and TYK2 inhibition, JAK2 inhibition seems a promising but not definitively tested mechanism for treatment of leukemia.<sup>1,15</sup> Enhanced protein tyrosine kinase (PTK) activity correlates with the development of cancer and other proliferative diseases. The hypothesis that PTK inhibitors may be of value in the treatment of cancer led to the systematic synthesis of selective tyrosine phosphorylation inhibitors (tyrphostins) that show *in vitro* and *in vivo* anticancer activity. Research efforts in the development of tyrphostins such as AG 957, AG 1112, and AG 1318 have been done; other tyrphostins are AG 1478 and RG 13022, which are both epidermal growth factor receptor kinase inhibitors; AG 490, a JAK2 kinase inhibitor; AG 1296, a PDGFR kinase inhibitor, and STI 571 (imatinib), already in clinical use.<sup>1</sup> It is clear that a better understanding of the molecular mechanisms of the diseases will lead into a better design of specific treatments.

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