original research report

# The mutation profile of *JAK2*, *MPL* and *CALR* in Mexican patients with Philadelphia chromosome-negative myeloproliferative neoplasms

Nancy Labastida-Mercado [a,b], Samantha Galindo-Becerra [a,b], Javier Garcés-Eisele [b,c], Perla Colunga-Pedraza [d], Valeria Guzman-Olvera [b], Virginia Reyes-Nuñez [c], Guillermo J Ruiz-Delgado [a,b,c], Guillermo J Ruiz-Argüelles [a,b,c,e,*]

[a] Centro de Hematología y Medicina Interna de Puebla, Mexico, [b] Universidad Popular Autónoma del Estado de Puebla, Mexico, [c] Laboratorios Clínicos de Puebla, Mexico, [d] Universidad Autónoma de Nuevo León, Mexico, [e] Universidad de Las Américas Puebla, Mexico

[*] Corresponding author at: Centro de Hematología y Medicina Interna de Puebla, 8B Sur 3710, 72530 Puebla, PUE, Mexico. Tel.: + 52 (222) 243 8100; fax: + 52 (222) 243 8428. · gruiz1@clinicaruiz.com · Received for publication 24 September 2014 · Accepted for publication 9 December 2014

Hematol Oncol Stem Cell Ther 2015; 8(1): 16–21


DOI: http://dx.doi.org/10.1016/j.hemonc.2014.12.002

**CONTEXT AND OBJECTIVE:** By using molecular markers, it is possible to gain information on both the classification and etiopathogenesis of chronic myeloproliferative neoplasias (MPN).

**METHODS:** In a group of 27 Mexican mestizo patients with MPNs, we studied seven molecular markers: the BCR/ABL1 fusion gene, the JAK2 V617F mutation, the JAK2 exon 12 mutations, the MPL W515L mutation, the MPL W515K mutation, and the calreticulin (CALR) exon 9 deletion or insertion. Patients with the BCR/ABL1 fusion gene were excluded. We studied 14 patients with essential thrombocythemia (ET), eight with polycythemia vera (PV), four with primary myelofibrosis (MF), and one with undifferentiated MPN.

**RESULTS:** We found twelve individuals with the JAK2 V617F mutation; five of them had been clinically classified as PV, five as ET, and one as MF. One patient with the MPL W515L was identified with a clinical picture of ET. Five patients with the CALR mutation were identified, four ET and one MF. No individuals with either the MPL W515K mutation or the JAK2 exon 12 mutations were identified. The most consistent relationship was that between PV and the JAK2 V617F mutation ($p = .01$).

**CONCLUSIONS:** Despite its small size, the study shows much less prevalence of JAK2 mutation in PV, ET and MF, which does not match international data.

**KEYWORDS:** Myeloproliferative neoplasms; Molecular markers; JAK2 V617F mutation; JAK2 exon 12 mutation; MPL W515L mutation; MPL W515K mutation; CAL-R mutations

## INTRODUCTION

Chronic myeloproliferative neoplasias (MPN) are clonal hematopoietic stem cell disorders characterized by proliferation of myeloid cell lineages in the bone marrow and increased numbers of mature and immature cells in the blood.[1,2] MPNs include polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (MF) and chronic myelogenous leukemia (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.[3,4] Although there are stringent diagnostic criteria for MPN subtypes, precise categorization remains a subject of debate and, furthermore, it can be difficult to differentiate some cases from reactive disorders. CML is characterized by a pathognomonic

molecular marker, the BCR-ABL1 fusion, and the primary abnormalities driving excess proliferation in most other cases have been obscure. Several lines of evidence have implicated aberrant protein kinase (PTK) signaling as the root cause of several MPNs.[3,4]

Mutations in JAK2, MPL and calreticulin (CALR) are highly relevant to the Philadelphia chromosome (Ph1)-negative myeloproliferative neoplasms. In 2005, the first recurrent molecular abnormality was described, consisting of a G > T point mutation in JAK2 (JAK2{NM_004972.2}:c.2343G > T) and resulting in a valine to phenylalanine substitution at residue 617 (V617F).[5] Subsequently, the discovery of mutations in MPL, represented by a W > L (MPL{NM_005373.2}:c.1544G > T) or W > K (MPL:c.1543_1544TG > AA) shift at codon 515, and of variable molecular abnormalities (point mutation, insertion, deletion) in JAK2 exon 12 was also reported.[6] Almost all patients with PV have a somatic genetic defect in JAK2 that is represented by the V617F allele in 90–95% of cases and by abnormalities in exon 12 in roughly 2%, while they are spared by MPL mutations. On the other hand, only 60% of patients with ET or MF harbor the JAK2 V617F mutation and 3–7% exhibit the MPL W515L/K mutation.[7] Recently, somatic mutations in the gene CALR, encoding calreticulin, have been found in most patients with ET or MF with nonmutated JAK2 and MPL. Mutant CALR is a result of frameshift mutations, caused by exon 9 deletions or insertions; type-1, 52-bp deletion (p.L367fs*46), and type-2, 5-bp TTGTC insertion (p.K385fs*47) variants constitute more than 80% of these mutations.[8]

With these molecular markers, it is possible to gain information on both the classification and etiopathogenesis of some MPNs. In a group of Mexican mestizo patients with MPNs, we studied seven molecular markers of the MPNs: the BCR/ABL1 fusion gene, the JAK2 V617F mutation, the JAK2 exon 12 mutations, the MPL W515L mutation, the MPL W515K mutation, the type-1 CALR mutation and the type-2 CALR mutation. We report here the results of these investigations.

## MATERIAL AND METHODS

### Patients

Patients with hematological malignancies who presented at the Centro de Hematología y Medicina Interna de Puebla after August 2005 were prospectively accrued in the study along with DNA samples from our bank. The diagnoses and classification of leukemia were done according to conventional criteria[1,9,10]; patients were studied, treated and followed up by one of the authors of this study (GJRA). Informed consent was obtained from all patients. The definition of mestizo employed in this study covers individuals born in Latin America who had both Amerindian and white ancestors.[11] Included in the study were 150 healthy blood donors as the control group.

### Molecular biology studies

*Analysis of the BCR/ABL1 fusion gene*

The BCR-ABL1 specific transcripts were detected by RT-PCR, as previously described.[12] Briefly, total RNA, purified from 1 ml peripheral blood, was reverse transcribed by standard methods. One fifth of the cDNA was amplified for 40 cycles (pre-cycle: 1 min 95 °C; cycles: 15 s 94 °C, 20 s 60 °C, 20 s 72 °C; post-cycle: 2 min 72 °C) in 50 µl final volume (2 U Taq, 1.5 mM MgCl2, 0.4 µM each primer: TCGTGTGTGAAACTCCAGAC, CCATCCCC CATTGTGATTAT, and ACTGCCCGGTTGTC GTGT). Ten microliters of amplified material were analyzed on 4.5% PAGE. Negative PCR results were validated by detection of ABL1 transcripts (primers: CCATCCCCCATTGTGATTAT and TAGCA TCTGACTTTGAGCCT).

*Analysis of the JAK2 V617F mutation*

An amplification refractory mutation system (ARMS) method was used according to Baxter et al.[13] 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 14 was amplified as an internal amplification control (364 bp), taking care not to exceed 0.05 µg of DNA per 50 µl amplification reaction. Amplification products were analyzed after electrophoresis on 4.5% polyacrylamide gels.

*Analysis of the MPL W5151K/L mutation*

For the detection of MPL mutations in separate reactions, ARMS primers were designed (W515 specific primer GGCCTGCTGCTGCTGAGATG, L515 specific primer GGCCTGCTGCTGCTGAGATT, K515 specific primer GGCCTGCTGCTGCTGAG GAA, and the common primer GGGCGGTATAG TGGGCGTGT) according to Newton et al.[14] with the introduction of extra destabilizing mismatches at position – 2 to increase specificity. Briefly, 10–250 ng of DNA was amplified (pre-cycle: 2 min at 94 °C; 35 cycles: 20 s at 94 °C, 30 s at 64 °C,