

## Original Article

# Evaluation of JAK2V617F mutation prevalence in myeloproliferative neoplasm by AS-RT-PCR

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## Abstract

### Objective

JAK2 is a non-receptor tyrosine kinase that plays a major role in myeloid disorders. JAK2V617F mutation is characterized by a G to T transverse at nucleotide 1849 in exon 12 of the JAK2 gene, located on the chromosome 9p, leading to a substitution of valine to phenylalanine at amino acid position 617 in the JAK2 protein.

### Methods

In this study we evaluated RNA from 89 patients with MPNs and statistical analysis done with Mann-Whitney test. The mutation detected by allele specific-PCR(AS-PCR). In addition, 3 samples were sequenced in Millegen company.

### Results

Using AS-PCR method 26/30 polycythemia vera patients (86%), 8/13 IMF patients (61%), 8/15 ET patients (53%) and none of 31 CML patients were positive for JAK2 V617F mutation.

Polycythemia vera patient carrying the mutation displayed higher levels of WBC (p=0.03). Sixteen of 26 JAK2V617F positive patients were female that demonstrate correlation between the presence of a mutant allele and sex. The differences in other groups were not significant.

### Conclusion

We have shown that a single acquired point mutation in JAK2 is present in virtually most patients with PV and in about half of those with either ET or IMF. However in other study the JAK2V617F mutation has been detected in the vast majority of patients with polycythemia vera (65-95%). It was less frequent in patients with essential thrombocythemia (23-57%), idiopathic myelofibrosis (23-57%) and chronic myeloid leukemia 19% (3/16 CML Ph-). Detection of the mutation is helpful in differential diagnosis, prognosis, and prediction of therapeutic response.

### Key words

JAK2 mutation, AS-RT-PCR, polycythemia vera, essential thrombocythemia, primary myelofibrosis.

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## Introduction

JAK2 V617F mutation in chronic myeloproliferative neoplasm was discovered by different methods (1&4). JAK2 is a cytoplasmic tyrosin kinase with a key role in a different signal transduction pathways like interleukin 3, 5 (IL3, IL5) and granulocyte-monocyte-colony stimulation factor (GM-CSF) (5&6). JAK2 contains four major function domains, tyrosin kinase domain (JH1), pseudo kinase domain (JH2), SH2 domain and the FERM domain.

Identification of the JAK2 mutation is new discovery in the field of chronic myeloproliferative neoplasm (MPNS) (7&8). Different techniques have been used for the detection of this mutation, such as genomic DNA-PCR-sequencing, RT-PCR, PCR-ARMS (amplification refractory mutation system), Allele-specific PCR (AS-PCR), PCR restriction analysis, and real-time PCR (9). In this study frequency of JAK2 mutation, in patients with essential thrombocythemia (ET), polycythemia vera (PV) and Idiopathic myelofibrosis (IMF) and chronic myelogenous leukemia (CML) was studied using AS-PCR method in order to detect the JAK2V617F.

## Materials and Methods

We evaluated 89 previously treated and newly diagnosed patients with MPDs and 50 normal controls. Thirty patients were diagnosed with PV, 13 patients with IMF, 15 patients with ET and 31 patients with CML.

The patients were selected from out patient clinic of Bone Marrow Transplantation Center of Shariati Hospital and the Oncology Center of Imam Khomeini Hospital. The mutation was detected by AS-RT-PCR on mononuclear cell (9).

Single stranded RNA (SsRNA) was extracted from 5ml of fresh peripheral blood in EDTA with standard trizol method (11). RNA is first reverse transcribed into cDNA using a reverse transcriptase (RT), the resulting cDNA is used as templates for subsequent PCR amplification.

AS-PCR is a common PCR technique widely used to detect known mutation based on the amplification of mutant alleles using mutant specific primer. This method was first reported by Baxter et al. (10) and sequences of the primers used in this study are given below (10):

Forward 5'- GAAGATTTGATATTTAATGAAAGCCTT-3'

Reverse 5'- GTAATACTAATGCCAGGATCACTAAGTT-3'

Mutant 5'- AGCATTGGTTTTAAATTATGGAGTATATT-3'

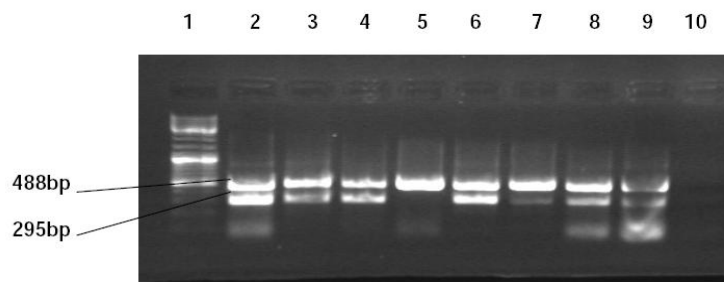
PCR parameters were as follow: Denaturation for 5 minutes at 95 °C, for one cycle; 36 cycles of denaturation at 95 °C for 30 seconds, annealing at 56 °C for 40 seconds, elongation at 72 °C for 45 seconds, hold for 10 minutes at 72 °C, and infinite hold at 4 °C.

A 488-bp product was obtained from both wild type and mutant alleles, while a 295 bp product indicated the presence of the mutation allele (4). Of course the PCR was not designed to distinguish zygosity. The zygosity could be determined by RFLP-PAGE (restriction fragment length polymorphism/polyacrylamide gel electrophoresis) sample interpretation (11).

## Results

In this study we evaluate JAK2V617F mutation in 89 MPDs patients. Fifty patients were male (56.6%) and 39 were female (43.4%) and the mean age was 48 ranging from 16 to 76 years, 86.6% (26/30) of those with polycythemia vera (PV) 53.3% (8/15) of those with essential thrombocythemia (ET), 61.5% (8/13) of those with idiopathic myelofibrosis (IMF) and none of 31 chronic myeloid leukemia (CML) patients had JAK2 gene mutation (Figure 1). In 3 random selected patients the mutation was confirmed by sequencing analysis on purified PCR product by Millegen company.

Fig1. AS-PCR assay for detection of JAK2V617F mutation; lane1, 100bp DNA ladder, lane2, positive control, lane3,4,6,8 mutation positive patients, lane5 mutation negative patients, Lane10 negative control.



Analyses in the polycythemia group of patients revealed that presence of mutation was correlated with older age, sex (16 of 26 were female), and higher level of White Blood Cell (WBC) count. splenomegaly was more frequent in the mutation positive patients (17 of 26) than the mutation negative patients. However, in other items not significant differences seen (table-1). The correlation was found between positive mutation and older age in patients with ET and IMF (table-2,3).

Tabel-1: Jak2 mutational status and laboratory feature in patients with polycythemia

Jak2 mutational status in patients with polycythemia vera	Jak2V617F	Jak2 wide- type	P
Total (percentage)	26(86%)	4(14%)	
Laboratory feature at diagnosis			
White cell count, $\times 10^3$ /L			0.03
Mean $\pm$ SD	22.26 $\pm$ 37.6	5.95 $\pm$ 1.43	
Median	12.05	5.85	
Hemoglobine level, g/L			
Mean $\pm$ SD	16.98 $\pm$ 1.805	18.3 $\pm$ 1.151	
Median	17.35	18.35	

Table-2 : Jak2 mutational status and laboratory feature in patients with Essential thrombocythemia

Jak2 mutational status in patients with Essential thrombocythemia	Jak2V617F	Jak2 wide- type	P
Total( percentage)	8(53.3)	7(46.7)	
Laboratory feature at diagnosis			
White cell count, $\times 10^3$ /L			0.065
Mean $\pm$ SD	8.41 $\pm$ 2.30	7.01 $\pm$ 5.32	
Median	8.7	5.50	
Platelet count, $\times 10^3$ /L			0.4
Mean $\pm$ SD	9.101 $\pm$ 1.99	9.78 $\pm$ 2.06	
Median	8.85	9.51	

Tabel-3: Jak2 mutational status and laboratory feature in patients with idiopathic myelofibrosis

Jak2 mutational status in patients with idiopathic myelofibrosis	Jak2V617F	Jak2 wide- type	P
Total (percentage)	8(61.5%)	5(38.5%)	
Laboratory feature at diagnosis			
White cell count, x10 <sup>3</sup> /L			
Mean± SD	12.4±6.99	5.92±2.05	0.1
Median	12.1	5.5	

## Discussion

JAK2 V617F mutation, in chronic myeloproliferative neoplasm, was investigated by different methods (1&4). Identification of the JAK2 mutation is new discovery in the field of chronic myeloid proliferative neoplasm (MPNS) (7&8). Different techniques have been used for this detection of the mutation, such as genomic DNA-PCR-sequencing, RT-PCR, ARMS -PCR, AS-PCR, PCR restriction analysis, and real-time PCR (9).

In Fruntz study, the prevalence of mutation in PV patients was 81% (58/72), in ET patients 41% (24/59) and in IMF patients 43% (15/53) (11). In Baxter et al's study in 2005 using mutation specific PCR method (4), the JAK2 mutation detected in 71 of 73 (97%) patients with PV, 29 out of 51 (57%) with ET, and 8 out of 16 (50%) with IMF.

In support of previous reports, we observed the mutation in 86% of PV patients which is comparable with result of James (86%) (1), Jelinek (86%) (14) and Jones studies (81%) (11). The highest rate of mutation (97%) has been reported by Lippert's group using allele-specific quantitative polymerase chain reaction (qPCR) (15) and the lowest rate (65%), has been reported by Kralovics, using DNA sequencing and microsatellite mapping (2). The highest detection rate in IMF was found by Jelink (14) with a frequency 95% (18 of 19 patients) who used pyrosequencing method. The lowest frequency was 35%, which was detected by Levin study (14). Campbell et al (4) found positive mutation in 53.4% patients using AS-PCR techniques.

AS-PCR was more sensitive than RFLP-PCR, pyrosequencing and ARMS for finding JAK2V617F mutation (11). The JAK2 mutation has not been found in our 31 CML patients, which is the same as Jones' group study (11), however they also detected JAK2 mutation and Abl-Bcr translocation in some patients who had received Imatinib treatment (16).

In summary, we have shown that a single acquired point mutation in JAK2 is present in virtually most patients with PV and in about half of those with either ET or IMF. Thus, the detection of mutation could be used not only as a diagnostic tool, but also for the classification and management of patients with MPDs.

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## Reference

- 1- James C, Ugo V, LeCouedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signaling causes polycythaemia vera. *Nature*. 2005jun; 434:1144–8.
- 2- Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005oct; 352:1779–90.

- 3- Levine RL, Wadleigh M, Cools J, Ebert BL. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*. 2005Nov; 7:387–397.
- 4- Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative diseases. *Lancet*. 2005; 365:1054–61.
- 5- Verma A, Kambhampati S, Parmar S, Plataniias LC. Jak family of kinases in cancer. *Cancer Metastasis Rev*. 2003; 22:423-434.
- 6- Parganas E, Wang D, Stravopodis D. Jak2 is essential for signaling through a variety of cytokine receptors. *Cell*. 1998; 93:385-395.
- 7- Huang LJ, Constantinescu SN, Lodish HF. The N-terminal domain of Janus kinase 2 is required for Golgi processing and cell surface expression of erythropoietin receptor. *Mol Cell*. 2001Ogu;8(6):1327–38.
- 8- Mcloman D ,Percy M ,McMullin M. JAK2V617F :A single mutation in the myeloproliferative group of disorders. *Ulster Med J*. 2006; 13(2) 112-119.
- 9- Speletas M ,Katodritou E. Correlations of JAK2V617F mutation with clinical and laboratory finding in patients with myeloproliferative disorders .*Leukemia*. 2007;1053-1062 .
- 10- Kannima S, Thongnoppakhun W, Chirayu U. Two-round allele specific-polymerase chain reaction: A simple and highly sensitive method for JAK2V617F: *Clinica Chimica Acta*. 2009; 401:148–151.
- 11-Christine F. Comparative Evaluation of three jak2V617F mutation detection methods. *American journal of clinical pathology*. 2007; 128: 847-865.
- 12- Jones AV, Kreil S, Zoi K, Waghorn K, Zhang L,Score J et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood*. 2005; 106:2162-2168
- 13- Jelinek J, Oki Y, Gharibyan V, et al. JAK2 mutation1849G > T is rare in acute leukemias but can be found in CMML,Philadelphia chromosome-negative CML and megakaryoblastic leukaemia. *Blood*. 2005; 106:3370-73.
- 14- Lippert E, Boissinot M, Kralovics R, et al. The JAK2-V617F mutation is frequently present at diagnosis in patients with essential thrombocythemia and polycythemia vera. *Blood*. 2006; 108: 1865–1867.
- 15- Hussein K, Bock O, Seegers A, Flasshove M, Henneke F, Buesche G, et al. Myelofibrosis evolving during imatinib treatment of a chronic myeloproliferative disease with coexisting BCR-ABL translocation and JAK2v617f mutation. *Blood*. 2007; 109: 4106-07.
- 16- Kais H, Bock O, Seegers A, Flasshove M, Henneke F, Buesche G, and Heinrich Kreipe H: Myelofibrosis evolving during imatinib treatment of a chronic myeloproliferative disease with coexisting BCR-ABL translocation and JAK2V617F mutation. *Blood*. 2007 may;109:4106-07.