

Evaluation of the JAK2 Gene Mutation in Moroccan Patients with Chronic Myeloproliferative Disorders

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ABSTRACT: The Janus kinase2 (JAK2) mutation has considerably helped understanding of the molecular pathogenesis of chronic myeloproliferative disorders (MPDs), hence this study investigated for the status of the JAK2 mutation among myeloproliferative disorders in Moroccan patients.

Mutation screening for JAK2 in patients with polycythemia vera, essential thrombocythemia and primary myelofibrosis was performed in 23 patients. The mutations were detected by allele specific-PCR (AS-PCR) and subsequently analyzed with PCR-sequencing. Of 23 patients, 14 (61%) patients had JAK2 mutation, whereas 9 (39%) patients were negative. The positive group included 75% patients with polycythemia vera, 38% patients with essential thrombocythemia, 67% patients with primary myelofibrosis.

JAK2-V617F mutation screening can be incorporated in the initial evaluation of patients suspected of having MPDs. The relationship between the JAK2 mutation and hematological indices can be used in new diagnostic and therapeutic strategies.

KEYWORDS: JAK2 mutation, AS-PCR, myeloproliferative disorders, Polycythemia vera, Essential thrombocythemia, Primary myelofibrosis.

I. INTRODUCTION

The myeloproliferative disorders (MPDs) comprise several clonal hematologic diseases that are thought to arise from a transformation in a hematopoietic stem cell; the cells in the bone marrow multiply in an uncontrolled way.

Chronic myeloid leukemia is a myeloproliferative disorder that is defined by its causative molecular lesion, the BCR-ABL fusion gene, which most commonly results from the Philadelphia translocation (Ph) [1]. The three main Ph-negative myeloproliferative disorders: Polycythemia vera (PV), Essential thrombocythemia (ET), Primary myelofibrosis (PMF) [2]. Less common conditions that are also considered myeloproliferative disorders under the classification used by the World Health Organization include systemic mastocytosis, chronic eosinophilic leukemia, chronic myelomonocytic leukemia, and chronic neutrophilic leukemia [3].

Many studies show the association of several chronic myeloid cancers with activated tyrosine kinases [4, 5]. The family of tyrosine kinases, termed Janus kinases (JAKs), has a vital role in signal transduction in cells. JAK2 is essential for the proliferation and differentiation of erythroid and megakaryocytic [6,7]. JAK2 plays a role in downstream signaling pathways, such as the JAK/STAT (signal transducer and activator of transcription) pathway that is involved in cytokine signaling. JAK2 possesses 7 defined regions of conserved homology, denoted as JAK homology (JH) domains 1–7. JH2 is a pseudokinase domain that early functional studies show has an inhibitory effect on the JAK2 kinase domain

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

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[8,9]. This newly identified somatic point mutation is a G-C to T-A transversion, resulting in the substitution of valine by phenylalanine at codon 617 (JAK2-V617F). In 2005, several groups reported a single, acquired mutation in the JAK2 gene on chromosome 9 in the majority of patients with PV, ET or PMF: 65-97% for PV, 23-57% for ET and 35-57% for PMF [10,11,12]. In this study, we evaluated the prevalence of the JAK2 mutation in patients with MPDs.

II. MATERIAL AND METHODS

1. Patients

Peripheral blood samples were obtained from 23 patients who attended several departments of internal medicine, hematology and gastrology in University Hospital Hassan II-Fez. The proportion of subtype of 23 MPDs patients was PV (n=12), ET (n=8), PMF (n=3). The necessary investigations, including measurements of white blood cell (WBC) count, red blood cell (RBC) count, platelet (Plt), hemoglobin (Hb), hematocrit (Hct), and clinical characteristics, were performed whenever required. Written informed consent was received from all participants.

2. DNA extraction

DNA was extracted from the blood samples collected in ethylene diamine tetraacetic acid anticoagulant with QiaAmp DNA Blood Mini kit, DNA was quantified using spectrophotometric measurements, and diluted with ddH₂O to a final concentration of 20 ng/ul.

3. JAK2-V617F Mutation Screening

All DNA samples were genotyped for the JAK2-V617F mutation by an allele-specific polymerase chain reaction (AS-PCR) and sequencing PCR.

4. AS-PCR for the detection of the JAK2-V617F Mutation

PCR was carried out on 25 µl reaction mixture using primers which were a common reverse primer (R)(5'-CTGAATAGTCCTACAGTGTTCAGTTTCA-3'), a forward internal control prime (FC)(5'-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3'), a forward mutant specific primer (FM)(5'-AGCATTTGGTTTTAAATTATGGAGTATATT-3'), and forward wild type specific primer (FW)(5'-ATCTATAGTCATGCTGAAAGTAGGAGA AAG-3'). two master mix is used: mix M contained 1 mmol/l Buffer, 2 mmol/l MgCl₂, 0.2 mmol/l of dNTP, 0.02 U of Taq DNA Polymerase (Invitrogen), 20 ng/ul of genomic DNA together with 1 µmol/l of R, 0.5 µmol/l of FC and 0.5 µmol /l of FM. Mix N contained the FW primer instead of the FM primer. The mix was subjected a Veriti™ 96-well Thermal Cycler 9902 (Applied Biosystems) for the gene amplification.

The PCR protocol included an initial denaturation of 95°C for 7 min, followed by 36 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Then, 10 µl volumes of the amplification product (Mix M and Mix N) were separated in a 2 % agarose-gel electrophoresis in TBE buffer containing 0.5 µg/l ethidium bromide, and visualized under ultraviolet light. Mutant and wild type products were 203 bp in length and internal control products were 364 bp. A 203 bp PCR product represented the presence of the mutant allele in Mix M and the wild type allele in Mix N (Figure 1).

International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 8, Issue 6, June 2019

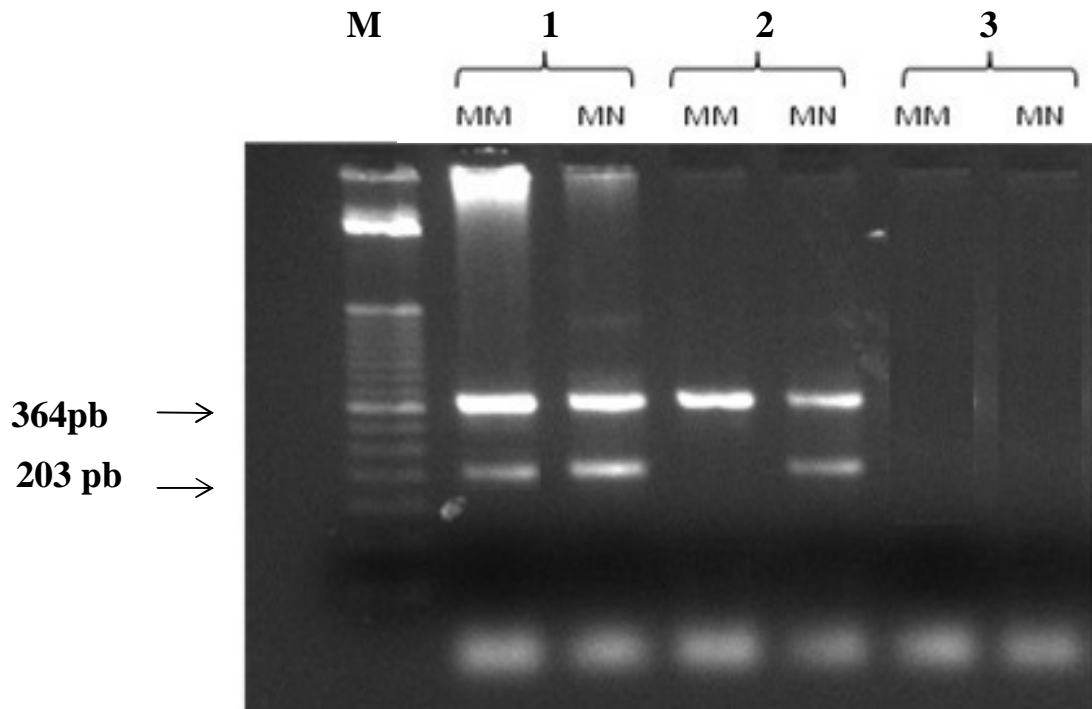


Fig 1: Agarose gel analysis for the detection of JAK2-V617F mutation in genomic DNA by Allele specific PCR. Lane M, 100 bp DNA ladder; lane1, mutation positive patient; lane2, mutation negative patient; Lane3 negative control.

5. PCR- sequencing for the detection of the JAK2-V617F Mutation

Samples were subsequently analyzed via PCR-sequencing. The PCR was performed in a 20 µl reaction mixture using a reverse primer (R) (5'-TCA TTGCTTTCCTTTTTCACAA-3'), a forward primer (F) (5'-GGGTTTCCTCAGAACGTT GA-3'). After the amplification, bidirectional direct of purified PCR products was performed using the BigDye Terminator V1.1 Cycle Sequencing Kit (ABI Prism™) and an Applied Biosystems 3500Dx Genetic Analyzer. The chromatogram was analyzed by the Sequencing Analysis SeqA v.5.4 (Applied Biosystems). And the sequences obtained have undergone Bioinformatics Analysis using "nucleotide blast" Alignment Program at <http://blast.ncbi.nlm.nih.gov> website.

III. EXPERIMENTAL RESULTS

As-Pcr analysis of JAK2-V617F mutation

Using the allele-specific PCR, Gel electrophoresis of PCR amplicons in a normal subject (wild type) and in representative patients with mutation are shown in Figure 1. A 364 bp (common, primers FC + R) product was obtained from both wild-type and mutation alleles. A 203 bp (primers FW + R) product was generated by JAK2 wild-type samples in Mix N. A 203 bp (primers FM + R) product by JAK2 mutation alleles in Mix M.

A positive heterozygous result was obtained for 12 patients, and in 2 PV cases sequencing analysis revealed a pathogenic mutation. Amplicons corresponding to the wild-type and JAK2-V617F mutation subjected to sequencing (Figure 2).

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Vol. 8, Issue 6, June 2019

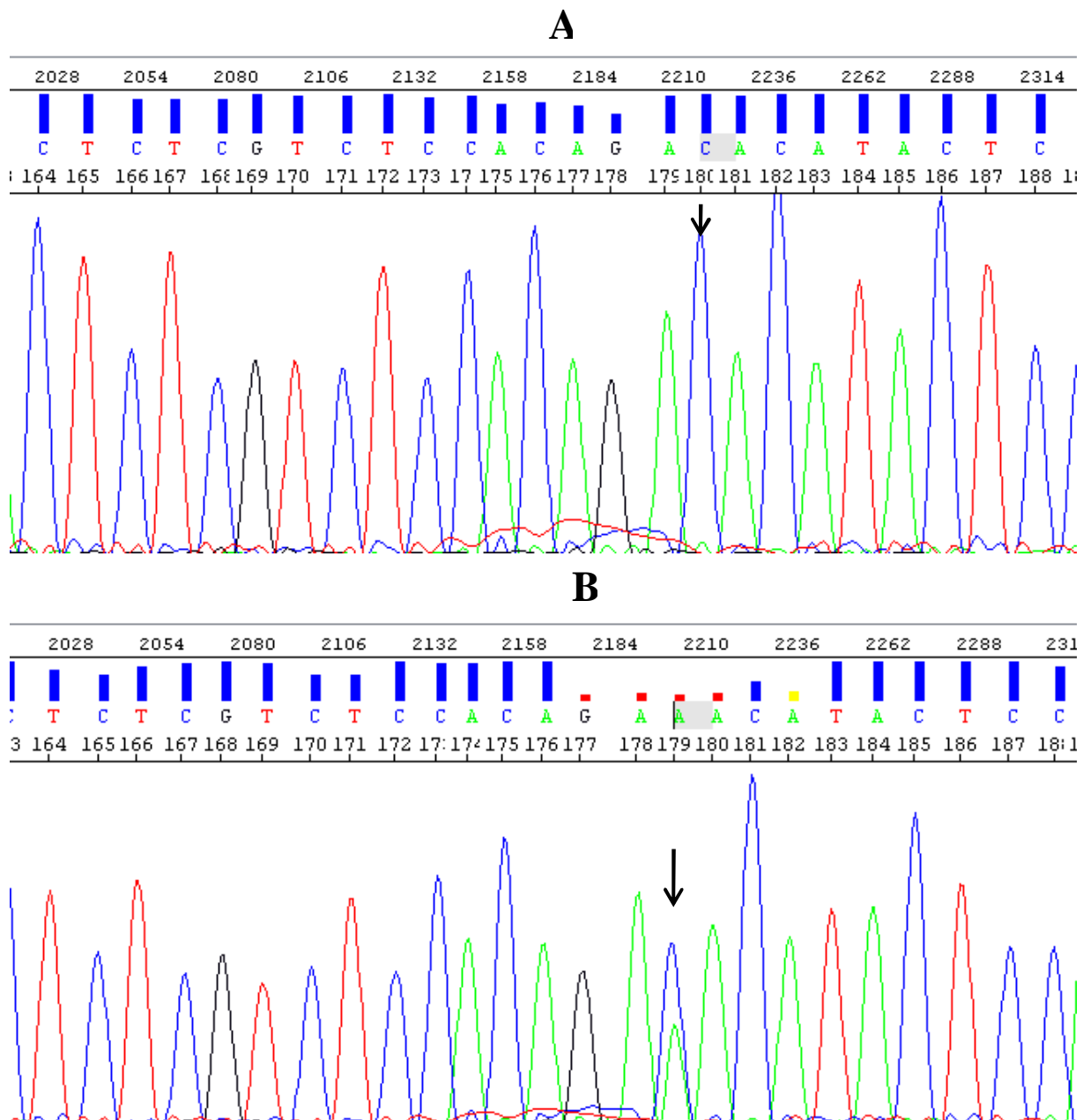


Fig 2: Sequencing chromatograms derived from the PCR sequencing products for: (A) a normal patient with wild-type sequence; (B) a heterozygous patient.

Prevalence of JAK2 mutation in patients with MPDs

In this study we evaluate JAK2 mutation in 23 MPDs patients. 16 patients were male (70%) and 7 were female (30%). In this group, 14 (61%) of the patients were positive for the JAK2 mutation, whereas 9 (39%) patients were negative. The proportion of positive cases per disease subtype ranged from 75% (9 of 12) for PV, 38% (3 of 8) for ET and 67% (2 of 3) for PMF.

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

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The mean age of JAK2-V617F positive MPDs patients was 55 years (range 26-77 years) and that of the wild type was 47.41 years (range 15-91 years).

Hematological indexes, including WBC count, RBC count, Hb, Hct and Plt count in the positive and negative groups are shown in Table 1 and 2.

Table 1: Hematological parameters in JAK2-positive and -negative patients.

		WBC	RBC	HB	HCT	Plt
Positive patients	PV					
	mean± SD	13,74 ± 5,16	7,65 ± 0,84	18,55 ± 1,20	57,06 ± 2,53	568 ± 269,93
	Median	13,96	7,98	18,2	56,1	600
	(range)	(6 - 24,36)	(6,43 - 8,61)	(17,4 - 21)	(54 - 61)	(275 - 1024)
	TE					
	mean± SD	26,97 ± 19,28	4,71 ± 1,73	13,86 ± 3,46	44,87 ± 11	721,68 ± 451,75
	Median	23,97	4,14	15,2	50,8	727,5
	(range)	(3,4 - 62,53)	(3,1 - 7,16)	(9 - 17,1)	(27,8 - 54)	(120,12 - 1428)
	PMF					
Negative patients	mean± SD	15,05 ± 8,56	3,45 ± 0,22	7,3 ± 2,12	29,25 ± 0,91	254,5 ± 75,66
	Median	15,05	3,45	7,3	29,25	254,5
	(range)	(9 - 21,1)	(3,29 - 3,6)	(5,8 - 8,8)	(28,6 - 29,9)	(201 - 308)
	PV					
	mean± SD	10,47 ± 10,12	6,34 ± 1,21	18,66 ± 2,15	56,68 ± 7,80	315,53 ± 352,76
	Median	7,25	6,21	18	53,6	245
	(range)	(4,2 - 42,9)	(5 - 9,15)	(17 - 24)	(47,5 - 74)	(96 - 1450)
	TE					
	mean± SD	8,96 ± 6,40	4,94 ± 0,98	13,23 ± 4,03	42,12 ± 8,52	465,43 ± 510,78
	Median	7,62	5,4	14	43	256
	(range)	(4,05 - 35,59)	(2,7 - 6,9)	(5,3 - 17,9)	(23,5 - 53)	(44 - 2320)
	PMF					
	mean± SD	24,58 ± 28,8	3,71 ± 1,17	11,12 ± 3,66	31,6 ± 10,12	502,12 ± 593,85
	Median	9,38	3,45	10,85	30,25	196,5
	(range)	(1,75 - 77,2)	(2,01 - 5,8)	(5,7 - 17,4)	(17,7 - 48,6)	(23 - 1640)

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Table 2: Comparison of hematological parameters between male and female patients with the JAK2 mutation.

	Male JAK2 ^{V617F} (+)	Male JAK2 ^{V617F} (-)	Female JAK2 ^{V617F} (+)	Female JAK2 ^{V617F} (-)
Age, mean± SD	57,2 ± 16,81	46,96 ± 17,7	57,83 ± 15,5	45,52 ± 15,99
(range)	(31 - 72)	(15 - 91)	(34 - 80)	(19 - 77)
WBC (×10⁹/l)				
mean± SD	25,34 ± 22,15	15,55 ± 18,56	15,56 ± 4,89	7,66 ± 2,92
Median	23,94	8,41	17,45	7,62
(range)	(6 - 23)	(2,3 - 77,2)	(5,8 - 21)	(1,75 - 13,52)
RBC				
mean± SD	5,61 ± 1,89	5,6 ± 1,42	6,32 ± 2,17	4,42 ± 1,12
Median	6,53	5,66	6,82	4,41
(range)	(3,2 - 7,2)	(2,01 - 9,15)	(3,1 - 8,61)	(2,7 - 6,45)
HB (g/dl)				
mean± SD	15,6 ± 3,44	15,5 ± 4,02	15,74 ± 6,83	12,66 ± 4,75
Median	17,1	17,7	14,5	13,6
(range)	(10,2 - 18,3)	(12,1 - 18,2)	(3,4 - 25)	(5,3 - 22)
HCT	51,96 ± 10,61			
mean ± SD		47,1 ± 11,11	48,45 ± 12,15	40,05 ± 13,13
Median	54	50,3	53,2	39,3
(range)	(34,2 - 61)	(17,7 - 52,9)	(27,8 - 55,8)	(21 - 72)
PLt (×10⁹/l)				
mean± SD	513,2 ± 359,89	318,69 ± 360,15	606,41 ± 369,89	526,12 ± 543,72
Median	600	295,5	773,5	411
(range)	(12,01 - 939)	(29 - 94)	(388 - 1428)	(23 - 2320)

IV. DISCUSSION

The implication of the JAK2-V617F mutation in the pathogenesis of the MPDs is confirmed, but recent studies report higher rates of the presence of this mutation. This is mainly related to the choice of the technique used, the AS-PCR technique being known to be the most sensitive (2 to 3%) for the detection of point mutations [10,13].

In this study, we assessed prevalence of JAK2 mutation on 23 patients in three groups: PV, ET and PMF. Accordingly we found a high prevalence of JAK2-V617F mutation in patients with PV (75%) compared with ET and PMF (38% and 67%, respectively). Lucia et al. [14] applying an AS-PCR technique to a population of 158 patients with MPDs, they obtained 90.2% of JAK2-V617F positive cases within PV. Speletas et al. [13] found positive mutation in 81.4% with PV of 166 patients with MPDs.

The most prevalent JAK2 mutation in PV (97%) was reported by Baxter et al.[10], where they used an allele-specific PCR method. This difference in relation to our results could be explained by the small size of our samples (23 cases of MPDs).

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(A High Impact Factor, Monthly, Peer Reviewed Journal)

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In our study, patients with PV showed a higher WBC count and platelets in patients with JAK2 positive mutation as compared to patients with JAK2 negative mutation. This finding is consistent with previous studies, such as that of Poopak et al. [15], and Speletas et al. [13].

RBC count and Hb were higher in JAK2-positive women than JAK2-negative women, but RBC count and Hb did not differ in JAK2-positive and JAK2-negative men. Hct and Plt count were higher in both JAK2-positive men and women than in the negative group.

In our series the frequency of the mutation in the case of PMF corresponds to 67%. This is relatively higher value compared to the results reported elsewhere including those of [10,16,17].

We have identified the JAK2-V617F mutation in approximately 38% of patients with ET. Similar studies reported different results, of Levine et al. (32%) [12]. Kralovics et al. (23%)[18] and Jelinek et al. (30%)[19]. Campbell et al. evaluated 806 patients with ET for JAK2 mutation in 2005 [20], they showed that 414 (53.4%) of the patients were positive for the JAK2 mutation and 362 (46.6%) were negative.

In our group of ET patients, the presence of mutation was associated with higher levels of WBC and platelets. Present study showed that ET group with positive JAK2-V617F mutation have relation with higher WBC count. Poopak et al study [15] showed similar result in Iranian patients with relation between WBC count and positive JAK2-V617F mutation. Unlike Sazawal et al who reported no association was found in ET with WBC count [21].

We found a relation between the presence of the JAK2-V617F mutation and old age at diagnosis, which is in accordance to previous studies concerning either patients with MPDs [17,22,23]. This could be due to an overestimation of the age onset of the disease, because of the difficulty to determine the exact age of the emergence of the disease especially for patients diagnosed at a late stage.

The JAK2-V617F mutation is mainly heterozygous, but it can also be found in a homozygous state [11,21,24]. In our study, we found both wild type and mutated alleles in all the patients presenting a mutated JAK2-V617F.

On the other hand, 7 of the patients did not have the JAK2 mutation. Furthermore, other mutations in other genes may also be responsible for these diseases such as MPL W515K and MPL W515L mutations identified recently in 5% of the PMF and in 1% of TE [25,26].

In summary, our findings has presented the importance of mutation JAK2-V617F in the initial evaluation of patients with suspected MPDs. JAK2 mutation analysis is a sensitive and simple test, relatively cost-effective for proving the nature of these diseases. Finally, detection of this mutation may have an important role in the treatment of patients who would respond to JAK2 inhibitor therapy.

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