

## Prevalence of JAK2 V617F mutation in Indian patients with chronic myeloproliferative disorders

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**Background & objectives:** The Janus-associated Kinase-2 mutation *JAK2 V617F* in chronic myeloproliferative disorders (CMPDs) has been described as a frequent genetic event in majority of patients with polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF). Its frequency varies in different populations but there are no data from India. We therefore, looked for *JAK2 V617F* mutation in Indian patients with chronic myeloproliferative disorders.

**Methods:** Mutation screening for *JAK2 V617F* in patients with polycythemia vera, essential thrombocythemia and idiopathic myelofibrosis was performed in 75 patients attending Haematology clinic in a tertiary care hospital in north India, by polymerase chain reaction and restriction enzyme-based assay.

**Results:** *JAK2 V617F* mutation was found in 51 of 75 cases (68%) of CMPD, 82 per cent in PV, 70 per cent in ET and 52 per cent of IMF. The presence of *JAK2 V617F* mutation was associated with a higher haemoglobin level ( $P<0.05$ ), a higher white blood cell count ( $P<0.01$ ) and higher age ( $P<0.05$ ).

**Interpretation & conclusion:** The *JAK2 V617F* mutation was detected in 86 per cent of patients with CMPD disorders. Peripheral blood mutation screening for *JAK2 V617F* can be incorporated into the initial evaluation of patients suspected to have CMPD.

**Key words** Janus Kinase-2 mutation - myeloproliferative disorders - PCR

Chronic myeloproliferative disorders (CMPDs) are clonal haematopoietic stem cell disorders and include the BCR-ABL negative CMPDs like polycythemia vera (PV), essential thrombocythemia (ET), idiopathic myelofibrosis (IMF) and chronic eosinophilic leukaemia (CEL)<sup>1,2</sup>. Although the Polycythemia Vera Study Group (PVSG) and WHO criteria<sup>3,4</sup> have been used for diagnosing the BCR-ABL negative CMPDs, precise categorization remains a subject of debate, and

it may be difficult to differentiate some cases from reactive disorders.

*JAK2* mutation has been shown to be associated with a wide spectrum of chronic myeloproliferative disorders<sup>5-9</sup>. 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*). It is a gain of function mutation in that it releases the auto-inhibitory action of JH2 and recruits

STAT (signal transducer and activator of transcription) in the complete absence or in presence of only trace quantities of haematopoietic growth factors<sup>10-12</sup>. To date, this particular mutation has not been detected in control subjects, confirming that the allele is not a common polymorphism in the general population. The identification of *JAK2* V617F mutation by different independent groups from the West<sup>3-7</sup> was a major breakthrough in the understanding of the pathogenesis of BCR-ABL negative CMPDs. These different groups reported a variable frequency of *JAK2* V617F mutation ranging from 65-97 per cent for PV, 23- 57 per cent for ET and 35-57 per cent for IMF<sup>2,5-7</sup>. Since, there is no study available from India, we sought to look for the frequency of *JAK2* V617F mutation in patients with CMPD and other haematological diseases.

### Material & Methods

All consecutive patients with BCR - ABL negative CMPDs who came to the OPD of Haematology Department, All India Institute of Medical Sciences (AIIMS), New Delhi, India between January 2006 and April 2008 were included in the study. Both newly diagnosed cases (n=64) and previously diagnosed cases on follow up (n=11) were included. Patients diagnosed before 2001 were classified using PVSG criteria<sup>3</sup> and those presenting thereafter were categorized using WHO 2001 criteria<sup>4</sup>. The necessary investigations including complete blood count, total red cell mass, arterial oxygen saturation, serum erythropoietin levels and bone marrow biopsy were done whenever required. The proportion of subtype of 75 CMPD patients was PV (n=34), essential thrombocythemia (ET) (n=10), idiopathic myelo fibrosis (IMF) (n=31).

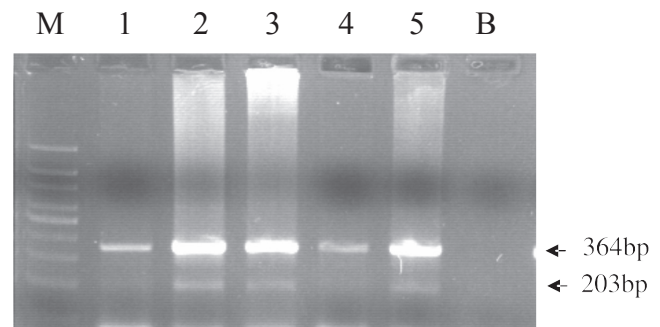
A total of 25 non CMPD patients were included as controls in the study; acute myeloid leukaemia (AML) (n=10), acute lymphoblastic leukaemia (ALL) (n=5) idiopathic thrombocytopenic purpura (n=5) and normal healthy controls (n=5).

Ethical approval for the study protocol was obtained, and written informed consent was taken from all patients.

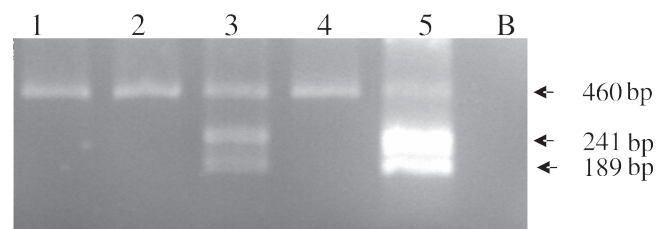
**Analysis of the *JAK2* V617F mutation:** Granulocytes were separated from peripheral blood (10 ml) and genomic DNA was extracted using conventional gradient centrifugation technique. All patients were routinely genotyped for the *JAK2* V617F mutation by an allele specific (ASO) polymerase chain reaction (PCR) exactly as described by Baxter *et al*<sup>5</sup> in which 1 µmol/l of a common reverse primer and 0.5 µmol/l

of one forward primer, specific for the mutant allele (giving a 203 bp product) and 0.5 µmol/l of another forward primer amplifying a 364 bp product from both mutant and wild type alleles which also serves as an internal PCR control were used (Fig. 1). Samples positive for the mutation were subsequently analyzed via polymerase chain reaction amplification and digestion (PCR-RFLP) with the restriction endonuclease BsaXI, (New England Biolabs, Hitchin, UK) which allows for an estimation between mutated and wild-type alleles<sup>5</sup>. Successful amplification was confirmed by electrophoresis on an ethidium bromide-impregnated 2 per cent agarose gel. The G-T mutation destroys a BsaXI site present in the wild type *JAK2* sequence (Fig. 2). This approach allows both normal and mutant alleles to be visualized and can distinguish between homozygous and heterozygous mutations.

**Statistical analysis:** Comparison of clinical characteristics between cases with and without *JAK2* V617F mutation was done by using Mann-Whitney U test (Wilcoxon rank sum test) for WBC, platelet count,



**Fig. 1.** Agarose gel analysis for the detection of *JAK2*V617F mutation in genomic DNA by Allele specific PCR. Lane M, 100-bp ladder; lanes 1-5, samples from patients, lanes 2, 3 and 5 show mutant band (203 bp) carried by the patients. Lanes 1 and 4 show a single band (364 bp) which is a wild type band and acts as an internal PCR control, lane B is the negative control. Arrows indicate the product of amplification.



**Fig. 2.** Restriction enzyme analysis of *JAK2*V617F mutation. Lanes 1-5, samples from patients, lane B is the negative control, lanes 1, 2 and 4 show homozygous mutant band at 460 bp as assessed by BsaXI restriction enzyme. Lanes 3 and 5 show the wild allele which was digested into 241 bp, 189 bp and 30 bp fragments.

splenomegaly, and disease duration; t-test for age and haemoglobin;  $\chi^2$  test for sex, and Fisher's exact test for thrombosis using software (SPSS, 11).  $P < 0.05$  was considered statistically significant.

### Results

*JAK2* V617F mutation was detected in 68 per cent (51 of 75) patients with chronic myeloproliferative disorders. The proportion of positive cases per disease subtype ranged from 82 per cent (28 of 34) for PV, 70 per cent (7 of 10) for ET and 52 per cent (16 of 31) for IMF. The mean age of *JAK2* V617F positive CMPD patients was  $53 \pm 11$  yr (range 28-73 yr) and that of the wild type was  $44 \pm 17.3$  yr (range 15-83 yr) ( $P < 0.05$ ). The overall presence of *JAK2* V617F mutation in CMPD was associated with a higher haemoglobin level

( $P < 0.05$ ), a higher white blood cell count ( $P < 0.01$ ) and higher age ( $P < 0.01$ ) (Table I). However, between specific CMPD disease subtypes, PV patients with *JAK2* V617F mutation displayed a significantly higher mean age ( $P < 0.05$ ) and a higher white blood cell count ( $P < 0.01$ ) (Table II). No association was found in ET and IMF patients. Patients with *JAK2* V617F mutation had higher splenomegaly (86%) as compared to the wild type (58%) but this was not statistically significant.

Previously diagnosed cases (PV=5, ET=4, MF=2) had a mean follow up of 2-12 yr. At the time of presentation their laboratory parameters were; Hb (mean=12.6 g/dl) TLC (median=20.3  $\times 10^9/l$ ) and platelet (median=6.29  $\times 10^9/l$ ). Patients with PV were on phlebotomy  $\pm$  hydroxyurea, ET patients were on

**Table I.** Clinical and laboratory features of patients (n=75) with chronic myeloproliferative disorders

	Disease subtype		<i>JAK2</i> V617F mutation		
	PV	ET	IMF	POS	NEG
No. of patients	34	10	31	51	24
Sex (male/female)	26/8	7/3	23/8	37/14	19/5
Age (yr), mean $\pm$ SD	$52 \pm 14.31$	$49 \pm 11.9$	$47 \pm 14.3$	$53 \pm 11.4^*$	$44 \pm 17.3$
Disease duration, months median (range)	8 (1-84)	7 (6-48)	6 (2-60)	7 (2-84)	6.5 (2-60)
Splenomegaly, %	75	86	78	86	58
Thrombosis/haemorrhage	1/3	2/1	0/0	2/3	1/1
WBC ( $\times 10^9/l$ ), median	29.9 (5.5-69.4)	18.0 (4.6-27.0)	11.5 (1.5-57.8)	24.8** (2.2-69.4)	12.5 (1.5-578.0)
Hb (g/dl), mean $\pm$ SD	$17.3 \pm 2.92$	$11.31 \pm 3.48$	$9.1 \pm 2.8$	$13.9 \pm 4.6^*$	$11.4 \pm 5.2$
Platelet ( $\times 10^9/l$ ), median	368.5 (72-950)	1347.5 (628-3242)	163.5 (13-1110)	362.5 (13-2240)	259.5 (50-3242)
<i>JAK2</i> V617F (+), n/N (%)	28/34 (82)	7/10 (70)	16/31 (52)		

*P* value refers to the comparison of *JAK2* V617F positive vs. negative subjects. PV, polycythemia vera; ET, essential thrombocythemia; IMF, idiopathic myelofibrosis; Disease duration, months from the original diagnosis to the time when blood sampling for this study was performed; WBC, white blood cell; Hb, haemoglobin, POS, positive; NEG, negative; N, number of patients; n, number positive  
 $P^* < 0.05$ ,  $** < 0.01$  compared to negative cases

**Table II.** Clinicohaematological parameters of *JAK2* positive and negative cases

Parameter	PV		ET		IMF	
	<i>JAK2</i> positive	<i>JAK2</i> negative	<i>JAK2</i> positive	<i>JAK2</i> negative	<i>JAK2</i> positive	<i>JAK2</i> negative
Number	28	6	7	3	16	15
Age (mean $\pm$ SD)	$53.19 \pm 10.07^*$	$39 \pm 23.66$	$53 \pm 9.81$	$40 \pm 13.23$	$49.88 \pm 13.27$	$46 \pm 15.54$
Hb (g/dl) (mean $\pm$ SD)	$16.19 \pm 2.94$	$17.15 \pm 2.95$	$12.83 \pm 5.22$	$11.5 \pm 11.13$	$9.75 \pm 2.36$	$8.47 \pm 3.22$
WBC ( $\times 10^9/l$ ), median	33.55 (22.2- 69.4)**	13.7 (5.5-23.6)	11.7 (4.6-27.0)	15.1 (6.2- 21.7)	12.6 (3.5-31.0)	9.8 (1.5- 57.8)
Platelet ( $\times 10^9/l$ ) median	368 (72.0-950)	320 (104-703)	1000 (628-2240)	1871 (1492-3242)	153 (13-847)	190 (50-1110)
Thrombosis	1	0	2	0	0	0
Haemorrhage	0	3	0	1	0	0

PV, polycythemia vera; ET, essential thrombocythemia; IMF, idiopathic myelofibrosis; WBC, white blood cell; Hb, haemoglobin  
 $P^* < 0.05$ ,  $** < 0.01$  compared to *JAK2* negative

aspirin ± hydroxyurea and MF patients were maintained on blood transfusions/hydroxyurea.

Thrombosis (n=3) or bleeding episode (n=4) was observed in four patients with PV of which 2 were positive for *JAK2* V617F mutation and in ET three patients were positive for *JAK2* V617F mutation. None of the cases with IMF experienced thrombotic or bleeding event.

Using the allele specific (ASO) technique and restriction enzyme analysis we observed that the overall frequency for homozygosity was higher than heterozygosity (73 vs 27%) respectively. The frequency of mutant alleles in the positive cases was found to be in accordance with the homozygous wild (HW) equilibrium and in negatives it was not determined as there was no mutation in the gene. It was found to be 82 per cent in PV, 71 per cent in ET and 56 per cent in IMF. The median duration of the disease in homozygous cases of PV and ET was 8 months (range 2-84 months) and that in heterozygous cases was 6 months (range 2-24 months). There was no significant difference between homozygous and heterozygous with any of the factors such as disease subtype, age, sex, splenomegaly, WBC count and haemoglobin level and platelet count. The *JAK2* V617F mutation status did not vary between the newly diagnosed and previously diagnosed patients.

### Discussion

In this study the *JAK2* V617F mutation was identified in majority of patients with PV (82%), ET (70%) and with IMF (52%). It was interesting to note that the frequency of *JAK2* V617F mutation in essential thrombocythemia was much higher (70%) than that reported in the West, but was comparable in patients with PV and IMF<sup>1,2,5,6</sup>. Our study showed a higher frequency of *JAK2* V617F mutation in homozygous state in patients with PV (82%) and ET (71%) than that reported from the West (PV, 25-30; ET, 2%)<sup>5-7</sup>. This difference could be attributed to ethnic variation but needs confirmation larger population studies from this region. Homozygosity implies to a higher allele burden, a condition where at least >50 per cent of *JAK2* alleles are found in granulocytes as a result of a mitotic recombination process rather than loss of heterozygosity<sup>1,2,5,6</sup>. It has been reported that the presence of homozygous mutation in PV and ET patients displays a higher leucocyte count and haematocrit value at diagnosis, and these patients present with larger spleen volume and are also older in age. They

are likely to display a symptomatic disease and a higher rate of evolution into secondary myelofibrosis<sup>10</sup>. In our study in PV and ET the mean age of homozygous cases was not significantly different from heterozygous cases suggesting that the mutation was acquired. The median duration of the disease in homozygous cases was 8 months (range 2-84 months) and that in heterozygous cases was 6 months (range 2-24 months). In previous studies, a time dependent increase in *JAK2* V617F allele burden in PV has been reported<sup>11</sup>. In our study we did not find any difference in disease duration between homozygous and heterozygous cases. Also, there was no difference found between *JAK2* V617F homozygous and heterozygous patients with any of the factors such as disease subtype, age, sex, splenomegaly, WBC count, haemoglobin level and platelet count which is at variance with other reported studies<sup>3,10</sup>. Since, homozygosity was predominant in our study in all the disease subtypes, it could be possible that being a developing country our patients present late in the course of the disease and thus they have a higher allele burden compared to that reported in the West.

Thrombotic episode or haemorrhage was observed in 7 patients of whom 5 were positive for the *JAK2* V617F mutation and were found to be only in homozygous state. *JAK2* V617F homozygosity has been associated with more frequent evolution into secondary myelofibrosis<sup>8</sup> and higher risk of thrombotic complication<sup>10</sup>. In our study two cases, one of PV and the other of ET showed evolution into myelofibrosis. They were both homozygous for the mutation. Our findings did not show any association between the presence of *JAK2* V617F mutation and risk of thrombosis or haemorrhage in CMPD patients since these events occurred in very few cases and this is consistent with the results from other studies<sup>12,13</sup>. Since, most of the patients of our study group had the *JAK2* V617F mutation in homozygous state these patients will need a longer follow up to see for evolution of the disease.

In summary, our findings support the recommendation that peripheral blood mutation screening for *JAK2* V617F be incorporated into the initial evaluation of patients with suspected CMPD. It is a sensitive and simple test, relatively cost-effective for proving clonality in these diseases. It also helps in excluding a large number of secondary causes. However, since the mutation may be absent in a few cases of PV, ET and IMF, it cannot be used as a single test for making the diagnosis. It should be done in addition to other tests like red cell mass, serum



erythropoietin, bone marrow biopsy before excluding diagnosis of suspected CMPD. *JAK2* V617F mutation screening can also be used for other indications such as unusual thrombotic complications like abdominal vein thrombosis, unexplained erythrocytosis and thrombocytosis. It is reiterated that as in the West, detection of this mutation may have a significant role in diagnosing BCR-ABL negative CMPD and in identification of subsets who would respond to *JAK2* inhibitor therapy.

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