

## Influence of JAK2V617F allele burden on clinical phenotype of polycythemia vera patients: A study from India

Sudha Sazawal, Kanwaljeet Singh, Sunita Chhikara, Rekha Chaubey, Manoranjan Mahapatra, Renu Saxena

## Abstract

**Background:** Elevated JAK2V617F allele burden is associated with enhanced expression of downstream target genes in Philadelphia negative chronic myeloproliferative neoplasms (CMPNs) which include PV, ET & PMF. Previous studies have shown the impact of JAK2V617F allele burden on clinical phenotype of CMPNs. However, there is no data from India regarding the association between JAK2V617F allele burden and clinical phenotype in PV. **Aims/Settings and Design:** We aimed to investigate the effect of allele burden on clinical phenotype in 90 JAK2V617F positive PV patients and to see its influence on disease related complications. **Material and Methods:** Allele burden of 90 JAK2V617F positive PV patients was quantified by Real-time polymerase chain reaction (RQ-PCR). **Results:** 74/90 (82.22%) were males and 16/90 (17.78%) were females (median 45 years, range 35-78). Patients with age >50 years had significantly higher JAK2V617F allele burden (median 40.15%, range 0.49–91.62 %) than patients with ≤ 50 years age (median 48.59 %, range 0.56–86.74 %;  $P < 0.032$ ). Patients with splenomegaly had significantly higher JAK2V617F allele burden (mean 50.24%, range 6.91–84.17%) than patients without splenomegaly (mean 33.82 %, range 0.49–71.83 %;  $P < 0.017$ ). Patients with higher allele burden (median 57.20, range 43.4–72.03%) had significantly raised thrombotic events than the patients with lower allele burden (median 37.38, range 0.49–84.17%;  $P < 0.043$ ). 49/90 (54%) were homozygous and 41/90 (46%) were heterozygous. **Conclusions:** Higher JAK2V617F allele burden showed association with increased age, splenomegaly and thrombotic events. Thus, it may be considered for prognostication and setting up the treatment protocol in PV patients.

**Key words:** JAK2V617F allele burden, polycythemia vera, real-time polymerase chain reaction

## Introduction

Philadelphia chromosome-negative chronic myeloproliferative neoplasms (CMPNs) are clonal hematopoietic disorders which include polycythemia vera (PV), primary myelofibrosis (PMF), and essential thrombocythemia (ET). A gain-of-function point mutation has been reported in the Janus 2 tyrosine kinase (JAK2) gene which increases the activity of JAK2 kinase and is known to affect the clinical outcome.<sup>[1,2]</sup> The JAK2V617F mutation is reported in approximately 90%–95% of PV patients and in approximately 50%–60% of ET and PMF patients.<sup>[3,4]</sup>

Mitotic recombination leads to homozygosity in JAK2V617F mutation and is present in about one-third of PV patients.<sup>[2-4]</sup> Previous studies have analyzed the impact of JAK2V617F mutation burden on disease phenotype by taking 50% as the cutoff value since homozygosity of JAK2V617F mutation was seen in patients with >50% allele burden.<sup>[5,6]</sup> However, due to the presence of mixed population of wild-type cells in the total cells harboring both homozygous and heterozygous mutation, the actual burden of homozygous JAK2V617F mutation can be under 50%.

Elevated JAK2V617F allele burden is associated with enhanced expression of downstream target genes and increased granulocyte activation.<sup>[5,7-10]</sup> Various studies have shown association between the allele burden and clinical phenotype.<sup>[11-13]</sup> The impact of JAK2V617F allele burden on several clinical parameters such as hemoglobin concentration, white blood cell (WBC) counts, platelet counts, and spleen size has been reported in CMPN patients.<sup>[11,12]</sup> In addition, the association between JAK2V617F allele burden and thrombotic risk has also been reported.<sup>[14,15]</sup>

Studies from India have reported the prevalence of JAK2 V617F mutation in CMPNs and correlated it with the clinicohematologic characteristics.<sup>[16,17]</sup> However, there is no data

regarding the association between JAK2V617F allele burden and clinical phenotype in PV.

In view of this, we sought to look for the effect of JAK2V617F allele burden on clinical phenotype in a study group of ninety JAK2V617F-positive PV patients and its influence on the disease-related complications.

## Materials and Methods

We analyzed ninety JAK2V617F-positive PV patients diagnosed at the Department of Hematology, AIIMS, New Delhi, India, between 2012 and 2016. All patients were categorized according to the WHO 2008 criteria. The general characteristics of the patients are shown in Table 1. CMPN patients other than PV were excluded from the study. Ethical approval for the study protocol was obtained and written informed consent was taken from all the patients.

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>[4]</sup> This approach allows both normal and mutant alleles to be visualized and can distinguish between homozygous and heterozygous mutations. These positive samples were further quantified for the allele burden of the JAK2V617F mutation using real-time PCR (RQ-PCR) with 7500 RT-PCR system (Applied Biosystems, Hayward, CA, USA). The RT-PCR was performed using TRUPCR JAK2V617F kit (3B BlackBio Biotech India Ltd., India). The TRUPCR JAK2V617F kit is based on standard curve method to quantify JAK2V617F allele burden. The RT-PCR mixture (20 µL final volume) contained 10 µl master mix, DNA template (total for about 100 ng), deionized water, 1 µl primer probe mix for wild type and

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Department of Hematology, All India Institute of Medical Sciences, New Delhi, India

**Correspondence to:** Dr. Sudha Sazawal,  
E-mail: sudha\_sazawal@hotmail.com

mutant allele detection. Amplification was performed by a standard protocol recommended by the manufacturer (94°C for 10 min; 40 repeated cycles of 94°C for 15s, 60°C for 60s). The reaction of each sample was run in triplicates. The JAK2V617F proportion was calculated from standard curve generated with wild-type JAK2 and JAK2 V617F standards.

The JAK2V617F mutant copy numbers (CNV617F) and JAK2 wild-type copy numbers (CNwt) obtained in the test results were used to calculate the JAK2V617F allele burden for the patient samples. We analyzed the JAK2V617F allele burden of the patients by taking >50% as the cutoff value for homozygosity and patients with allele burden ≤50% were taken as heterozygous.

$$\text{JAK 2 V617F allele burden \%} = \frac{\text{CNV617F}}{\text{CNV617F} + \text{CNwt}} \times 100$$

### Statistical analysis

Numerical variables were tested for normal distribution with the Kolmogorov–Smirnov test. Data were expressed as mean ± standard deviation. The nonparametric Pearson's product-moment correlation analysis was used to test for the relationship between JAK2V617F allele burden and different clinical variables. All tests for statistical significance were two tailed and  $P < 0.05$  was considered statistically significant.

### Results

Patient characteristics of all the ninety JAK2V617F-positive PV patients at diagnosis are summarized in Table 1. Of the ninety patients, 74 (82.22%) were males and 16 (17.78%) were females. The patients were divided into two groups based on age; 35 (38.80%) patients were ≤50 years and 55 (61.20%) patients were >50 years. Patients with age >50 years had significantly higher JAK2V617F allele burden (median 40.15%, range 0.49%–91.62%) as compared to patients with age ≤50 years (median 48.59%, range 0.56%–86.74%;  $P < 0.032$ ) [Table 1].

In all the patients, hemoglobin and hematocrit were >15 g/dl and 45%, respectively. There were 17 (18.89%) patients with WBC ≤10 × 10<sup>9</sup>/L and 73 (81.11%) patients with WBC >10 × 10<sup>9</sup>/L (median 17.60 × 10<sup>9</sup>/L, range: 4.7–55.4 × 10<sup>9</sup>/L). The patients with leukocytosis had higher JAK2V617F allele burden than the patients with no leukocytosis ( $P < 0.090$ ), but was not statistically significant. There were 29 (32.22%) patients with platelet count of ≤300 × 10<sup>9</sup>/L and 61 (67.78%) patients with platelet count >300 × 10<sup>9</sup>/L (median 436 × 10<sup>9</sup>/L, range: 3.3–1241 × 10<sup>9</sup>/L).

Spleen size was available in 52/90 patients. Of the 52 patients, 31 (59.62%) had splenomegaly at the time of diagnosis and 21 (40.38%) were negative for splenomegaly. Patients with splenomegaly had significantly higher JAK2V617F allele burden (mean 50.24%, range: 6.91%–84.17%) as compared to patients without splenomegaly (mean 33.82%, range: 0.49%–71.83%;  $P < 0.017$ ). History of thrombosis was available in 45/90 patients. Ten (22.44%) patients had been recorded for thrombotic events and 35 (77.56%) patients had absence of thrombosis. Patients with higher allele burden (median 57.20, range: 43.4%–72.03%) had significantly raised thrombotic events as compared to patients with lower allele burden (median 37.38, range: 49%–84.17%;  $P < 0.043$ ) [Table 1].

Out of the ninety patients, 49 were homozygous and 41 were heterozygous by ASO-PCR. A total of 32/49 (66%) patients

had allele burden >50% which was confirmed by RT-PCR. However, 17/49 (34%) patients who were homozygous by ASO-PCR had allele burden <50% which may be due to mixed population of wild-type allele along with the mutant allele, thus leading to reduction of allele burden <50%. The proportion of JAK2V617F allele burden is shown in Table 2.

### Discussion

The identification of JAK2V617F mutation is included as genetic diagnostic criteria for classification of CMPN and has been a cornerstone in understanding the pathophysiology of Philadelphia-negative CMPNs. A study by Scott *et al.* reported that JAK2V617F mutation did not occur in nonhematological cancers and was rare in myeloid malignancies other than the classic Philadelphia chromosome-negative CMPNs.<sup>[18]</sup> Studies have shown that in CMPN patients, the increase of JAK2V617F allele burden has a major influence on prognostic variables and clinical phenotype of the disease.<sup>[11,13]</sup>

Elevated JAK2V617F allele burden has been found to be associated with higher risk of myelofibrosis, increased hemoglobin levels, and systemic symptoms. However, its effect on WBC counts, splenomegaly, and thrombotic risk is controversial.<sup>[11,13]</sup> Previous reports have suggested that JAK2V617F allele burden could be related to spleen size and WBC count and is quite variable in Philadelphia chromosome-negative CMPN patients, which in part contributes to the clinical course of CMPN.<sup>[19,20]</sup>

**Table 1: Clinical characteristic of ninety patients with JAK2V617F mutation**

Variables	n=90 (%)	JAK2V617F burden (Median, %)	P
Age, Years			
≤50	35 (38.8)	40.15 (.56-86.74)	0.0327
≥50	55 (61.1)	48.59 (0.49-91.62)	
Gender			
Male	74 (82.22)	43.64 (0.49-86.74)	0.6311
Female	16 (17.78)	34.76 (17.24-91.62)	
Hemoglobin (g/dl)			-
≤15	90 (100)	43.49 (0.49-91.62)	-
≥15	0		
HCT (%)			-
≤45	0	43.9 (0.49-91.62)	-
≥45	90		
WBC (×10 <sup>9</sup> /L)			
≤10	17 (18.89)	37.05 (0.49-91.62)	0.090
≥10	73 (81.11)	46.72 (1.31-89.26)	
PLT(×10 <sup>9</sup> /L)			
≤300	29 (32.22)	49.18 (0.49-89.26)	0.508
≥300	61 (67.78)	40.52 (0.56-91.62)	
Splenomegaly			0.017
Yes	31 (52,59.62)	50.43 (6.91-84.17)	0.017
No	21 (52,40.38)	32.4 (0.49-71.83)	
Thrombosis			0.043
Yes	10 (45,22.44)	57.20 (43.4-72.03)	0.043
No	35 (45,77.56)	37.38 (0.49-84.17)	

**Table 2: JAK2V617F allele burden in polycythemia vera**

Allele burden	n=90 (%)	Mean±SD
0.1-20%	13 (14.44)	8.6±7.47
21-40%	22 (24.44)	27.96±5.46
41-60%	31 (34.44)	48.76±5.46
61-80%	20 (22.22)	66.06±4.8
81-100%	4 (4.44)	87.94±3.2

In this study, we investigated JAK2V617F allele burden in PV patients and correlated it with the clinical phenotype. Our study confirmed a significant correlation of the JAK2V617F allele burden with increased age, splenomegaly, and thrombosis. PV patients with age >50 years had significantly higher JAK2V617F allele burden when compared to patients with age ≤50 years ( $P < 0.032$ ). This is in line with a study from the West by Tefferi *et al.*; however, Zhao *et al.* did not find any effect of age in Chinese population.<sup>[8,21]</sup>

We observed that the patients with splenomegaly had significantly higher JAK2V617F allele burden as compared to patients without splenomegaly ( $P < 0.017$ ). Our results are in concordance with the study conducted by Vannucchi *et al.* in Italian population and Zhao *et al.* in Chinese population.<sup>[11,21]</sup> However, Tefferi *et al.* did not find any correlation of allele burden with splenomegaly.<sup>[12]</sup>

The impact of JAK2V617F mutation on thrombosis is reported to be controversial in MPN patients. Vannucchi *et al.* reported that JAK2V617F mutation had a significant association with thrombosis.<sup>[22]</sup> However, Passamonti *et al.* showed nonsignificant correlation with the risk of thrombosis in Italian PV patients.<sup>[20]</sup> In our study, we observed that patients with higher allele burden had significantly raised thrombotic events as compared to patients with lower allele burden ( $P < 0.043$ ).

Regarding influence of the JAK2V617F allele burden on gender, studies have reported that male PV patients had higher allele burden than females.<sup>[5,20]</sup> However, we did not find any significant correlation of gender with allele burden, which is confirmed by Zhao *et al.* in Chinese patients.<sup>[21]</sup>

We found that the patients with leukocytosis had higher JAK2V617F allele burden than the patients without leukocytosis ( $P = 0.090$ ), though not statistically significant. This is in contrast to a study from the West by Tefferi *et al.* who found significant correlation of leukocytosis with the allele burden.<sup>[12]</sup>

## Conclusion

JAK2V617F allele burden correlated with the phenotypic presentation of PV such as increased age, splenomegaly, and thrombosis. Thus, it may be considered for prognostication and setting up the treatment protocol in PV patients. Larger studies are needed to confirm these observations.

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## Conflicts of interest

There are no conflicts of interest.

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