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Correlations between *Janus Kinase 2* V617F Allele Burdens and Clinicohematologic Parameters in Myeloproliferative Neoplasms

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Background: This study evaluated potential correlations between the allele burden of the *Janus kinase 2 (JAK2)* V617F mutation and clinicohematologic characteristics in patients with myeloproliferative neoplasms (MPN).

Methods: Clinical and hematologic features were reviewed for 103 MPN patients, including patients with polycythemia vera (PV, 22 patients), essential thrombocythemia (ET, 64 patients), and primary myelofibrosis (PMF, 17 patients). *JAK2* V617F allele status and allele burdens were measured by allele-specific PCR and pyrosequencing, respectively.

Results: The *JAK2* V617F mutation was detected in 95.5%, 68.8%, and 52.9% of PV, ET, and PMF patients, respectively. *JAK2* V617F-positive ET patients were significantly older and exhibited higher neutrophil fractions, a higher frequency of thrombotic events, and a higher myelofibrosis rate than *JAK2* V617F-negative patients ($P < 0.05$). PV patients carried the highest mean T allele burden (66.0% ± 24.9%) compared with ET (40.5% ± 25.2%) and PMF patients (31.5% ± 37.0%) ($P = 0.00$). No significant correlations were detected between V617F allele burden and patient age, white blood cell count, Hb, Hct, or the platelet count for PV, ET, or PMF patients. ET patients with organomegaly had a higher *JAK2* V617F allele burden (53.4% ± 23.7%) than patients without organomegaly (35.6% ± 24.3%) ($P = 0.03$).

Conclusions: The *JAK2* V617F mutational status and its allele burden correlate with the clinicohematologic phenotypes of ET patients, including older age, higher neutrophil count, and greater rates of organomegaly, thrombotic events, and myelofibrosis. For PV and PMF patients, larger-scale studies involving more MPN patients are needed.

Key Words: *Janus kinase* 2, Allele, Myeloproliferative neoplasm

INTRODUCTION

The somatic mutation V617F in *Janus kinase 2* (*JAK2*) is an established diagnostic marker in *BCR-ABL1*-negative myeloproliferative neoplasms (MPN), e.g., polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [1-3]. However, correlations among the mutational status or allele burden of *JAK2* V617F and clinicohematologic characteristics of these diseases remain undetermined. Several retrospective studies have evaluated correlations between the *JAK2* V617F mutation and parameters such as age, white blood cell (WBC) count, Hb concentration, Hct, spleen size, disease duration, pruritus, and fibrosis in subjects with MPN; however, the results of these studies have been inconsistent [4-18]. The present

study was undertaken to: (1) determine whether MPN patients harboring the *JAK2* V617F mutation exhibit different clinicohematologic characteristics than patients lacking it; and (2) identify whether the allele burden of the *JAK2* V617F mutation is associated with the severity of clinicohematologic features of MPN.

METHODS

1. Study population and sample collection
In this study, we used stored bone marrow (BM) cells of 103 patients who were diagnosed with MPN during 2005-2010. Clinicohematologic data obtained from patient records and BM histology were reviewed. Hematological diagnoses and subtyping results were reconfirmed according to the 2008 WHO criteria [1]. Patient data included the following major thrombotic events: ischemic stroke, transient ischemic attack, myocardial infarction, angina pectoris, and deep vein thrombosis. Karyotyping and reverse transcription-PCR (RT-PCR) for the *BCR-ABL1* rearrangement confirmed that no patients harbored the *BCR-ABL1* fusion gene. This study was approved by the Institutional Review Board of the Dongsan Medical Center.

2. Analysis of *JAK2* V617F mutation and allele burden
Each patient's *JAK2* V617F mutation status (n=103) was determined by allele-specific PCR, according to the manufacturer's instructions (Seegene, Seoul, Korea). For patients with sufficient stored BM samples (n=85), *JAK2* V617F allele burdens were determined by pyrosequencing. Briefly, DNA was isolated using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) and amplified using the following biotin-labeled primers: forward 5′-GAAGCAGCAAGTATGATGAGCA-3′; reverse 5′-TGCTCTGAGAAAGGCATTAGAA-3′. Single-stranded biotinylated templates were then isolated and sequenced using the sequencing primer 5′-TTACTTACTCTCGTCTCCAC-3′. All primers were designed using the Pyromark Assay Design 2.0 software (Qiagen). Percentages of *JAK2* V617F mutant alleles were determined using the Allele Frequency Quantification function in PyroMark Q24 Software 2.0, according to the manufacturer's specifications (Qiagen).

3. Statistical analysis
Correlations between the mutational status of *JAK2* V617F and WBC count, neutrophil fraction, Hb, Hct, or platelet count were analyzed using independent T-tests. Frequency differences of sex, organomegaly, thrombotic event, or myelofibrosis according to the *JAK2* V617F mutational status were assessed using a Chi-square or Fisher's exact test. Correlations between clinicohema-

tologic parameters and *JAK2* V617F allele burden were examined using ANOVA or independent-T-tests. All analyses were conducted using SPSS v19.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was assigned at $P < 0.05$.

RESULTS

1. Clinicohematologic findings by MPN subtype
All 103 MPN patients (53 men, 50 women; mean age 64.0 ± 12.8) were diagnosed with chronic-phase MPN with the following subtypes: 22 PV (mean age 62.2 ± 11.6), 64 ET (mean age 63.8 ± 13.8), and 17 PMF (mean age 63.8 ± 13.8) (Table 1). The mean age of patients across MPN subgroups was not statistically different. Mean WBC counts and neutrophil fractions were statistically similar across MPN subgroups, except for a significantly higher neutrophil fraction in PV patients compared with PMF patients ($P=0.01$). Mean Hct and Hb concentrations significantly differed by disease subgroup, with PV patients exhibiting the highest values, followed by ET and PMF patients, in that order ($P=0.00$). Mean platelet counts were highest in ET patients ($P=0.00$) and did not differ significantly between PV and PMF patients. Organomegaly, including splenomegaly and hepatomegaly, was most frequent in PMF patients (64.7%) compared with ET (23.4%) or PV patients (31.8%). Thrombotic events occurred most frequently in ET patients (34.4%) and least frequently in PV patients (13.6%). Myelofibrosis was detected in all PMF patients, in 73.7% of PV patients, and in 72.9% of ET patients. Severe-grade myelofibrosis occurred most frequently among PMF patients (46.7%).

2. *JAK2* V617F status and clinicohematologic findings
The *JAK2* V617F mutation was detected in 74 of 103 patients (71.8%). Subtype-specific *JAK2* V617F detection rates were 95.5% for PV patients, 68.8% for ET patients, and 52.9% for PMF patients (Table 2). When clinicohematologic data were compared between *JAK2* V617F-positive and V617F-negative patients in the PV and PMF subgroups, no significant differences were detected. In contrast, *JAK2* V617F-positive ET patients were significantly older and displayed higher neutrophil fractions, higher frequencies of thrombotic events, and higher myelofibrosis rates than *JAK2* V617F-negative ET patients.

3. *JAK2* V617F allele burden and clinicohematologic findings
JAK2 V617F allele burdens could be measured for 85 of the 103 patients in the study population. This corresponded to 22 PV patients, 47 ET patients, and 16 PMF patients (Table 3). The PV

Table 1. Clinicohematologic characteristics according to myeloproliferative neoplasm (MPN) subtypes

	PV	ET	PMF	Total
N of patients (Male : Female)	22 (9 : 13)	64 (33 : 31)	17 (11 : 6)	103 (53 : 50)
Age (yr, mean ± SD)	62.2 ± 11.6	63.8 ± 13.8	67.2 ± 10.0	64.0 ± 12.8
WBC ($\times 10^9$/L, mean ± SD)	18.4 ± 10.4	17.0 ± 17.9	21.8 ± 19.6	18.1 ± 16.8
Neutrophil (%, mean ± SD)	76.4 ± 11.4	69.2 ± 15.6	62.3 ± 18.7	69.6 ± 15.8
Hct (%, mean ± SD)	54.0 ± 11.0	41.5 ± 7.5	27.1 ± 9.2	41.9 ± 11.8
Hb (g/dL, mean ± SD)	18.3 ± 2.7	13.1 ± 2.9	9.0 ± 2.9	13.5 ± 4.0
Platelet ($\times 10^9$/L, mean ± SD)	432.3 ± 227.3	1,091.4 ± 387.8	324.4 ± 313.1	828.9 ± 487.2
Organomegaly (%)	31.8	23.4	64.7	32.0
Thrombotic event (%)	13.6	34.4	17.7	27.2
Myelofibrosis (%)				
Mild	42.1	50.8	33.3	46.24
Moderate	21.1	13.6	20.0	16.1
severe	10.5	8.5	46.7	15.1
Total positive	73.7	72.9	100.0	77.4
N of *JAK2* V617F-positive (%)	21 (95.5)	44 (68.8)	9 (52.9)	74 (71.8)

Abbreviations: PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; WBC, white blood cell.

Table 2. Clinicohematologic characteristics according to *Janus kinase 2* (*JAK2*) mutation status

	PV		ET			PMF		
	JAK2 V617F	*JAK2* wild-type	*JAK2* V617F	*JAK2* wild-type	P*	*JAK2* V617F	*JAK2* wild-type	P*
N of patients (%)	21 (95.5)	1 (4.5)	44 (68.8)	20 (31.2)		9 (52.9)	8 (47.1)	
Male : Female (N)	8 : 13	1 : 0	25 : 19	8 : 12	0.28	5 : 4	6 : 2	0.62
Age (yr, mean ± SD)	62.9 ± 10.7	41.0	66.6 ± 12.7	57.8 ± 14.7	0.03	68.7 ± 9.0	65.5 ± 11.5	0.53
WBC ($\times 10^9$/L, mean ± SD)	18.9 ± 10.4	8.1	19.1 ± 19.6	12.4 ± 12.8	0.17	19.7 ± 13.1	23.8 ± 25.3	0.70
Neutrophil (%, mean ± SD)	77.5 ± 10.4	52.8	72.2 ± 16.0	62.5 ± 12.0	0.02	68.6 ± 10.1	56.1 ± 23.6	0.20
Hct (%, mean ± SD)	53.7 ± 11.2	59.2	41.1 ± 7.3	42.2 ± 8.0	0.63	28.5 ± 11.7	25.7 ± 6.4	0.57
Hb (g/dL, mean ± SD)	18.2 ± 2.7	20.2	12.9 ± 3.0	13.3 ± 2.7	0.69	9.3 ± 3.6	8.6 ± 2.1	0.65
Platelet ($\times 10^9$/L, mean ± SD)	438.3 ± 231.1	306.0	1,111.5 ± 345.2	1,038.5 ± 474.0	0.47	440.4 ± 392.6	208.4 ± 158.5	0.16
Organomegaly (%)	33.3	0.0	29.5	10.0	0.18[†]	77.8	50.0	0.34[†]
Thrombotic event (%)	14.3	0.0	45.5	10.0	0.01[†]	33.3	0.0	0.21[†]
Myelofibrosis (%)								
Negative	39.9	0.0	17.1	50.0	0.02[†]	0.0	0.0	-
Mild	22.2	0.0	53.7	44.4		28.6	37.5	
Moderate	11.1	0.0	19.5	0.0		14.3	25.0	
Severe	72.2	0.0	9.8	5.6		57.1	37.5	

*P values describe differences between *JAK2* V617F and *JAK2* wild-type by independent T-test or [†]Chi-square test.
Abbreviations: PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; WBC, white blood cell.

subgroup carried the highest mean *JAK2* V617F allele burden (66.0% ± 24.9%) compared with ET (40.5% ± 25.2%) and PMF patients (31.5% ± 37.0%) (P = 0.00). To examine the relationship between clinicohematologic parameters and V617F allele bur-

dens, PV, ET, and PMF patients were stratified by V617F allele burden as follows: 0-25%, 26-50%, 51-75%, or 76-100%. PV patients were most frequently classified into the 76-100% allele group (50.0%), whereas ET and PMF patients more often popu-

Table 3. Associations of clinicohematologic parameters with *Janus kinase 2* (*JAK2*) V617F allele burden

T allele burden	PV (N=22)	P^*	ET (N=47)	P^*	PMF (N=16)	P^*	Total (N=85)	P^*
Mean±SD	66.0±24.9		40.5±25.2		31.5±37.0		45.4±30.1	
N of patients (%)								
0-25%	2 (9.1)		10 (21.3)		9 (56.3)		21 (24.7)	
26-50%	4 (18.2)		24 (51.1)		3 (18.8)		31 (36.5)	
51-75%	5 (22.7)		6 (12.8)		0 (0.0)		11 (12.9)	
75-100%	11 (50.0)		7 (14.9)		4 (25.0)		22 (25.9)	
Age (yr, mean±SD)								
0-25%	59.0±11.3	0.35	56.1±19.8	0.06	64.4±10.7	0.43	59.0±16.0	0.06
26-50%	63.5±10.8		69.8±7.5		64.7±13.3		68.5±8.5	
51-75%	66.7±9.5		65.8±10.2				66.2±9.4	
75-100%	62.0±12.4		63.1±17.7		72.3±2.1		64.2±13.4	
WBC (×10⁹/L, mean±SD)								
0-25%	11.1±4.2	0.20	15.3±17.6	0.51	25,511±2,3896	0.84	19.3±20.0	0.47
26-50%	16.8±1.9		19.6±24.1		20,523±13,411		19.4±21.4	
51-75%	12.8±6.0		14.8±85.7				13.9±7.2	
75-100%	22.9±12.6		29.2±15.0		17,730±12,736		24.3±13.4	
Neutrophil (%, mean±SD)								
0-25%	59.7±9.8*	0.06	69.1±10.5	0.47	60.0±20.5	0.48	64.3±15.6	0.01
26-50%	75.5±10.0		70.5±20.2		71.7±6.7		71.2±18.1	
51-75%	72.8±15.7		75.1±9.3				74.1±12.0	
75-100%	81.3±6.8		80.2±7.6		71.1±8.3		79.5±7.7	
Hct (%, mean±SD)								
0-25%	54.1±7.2	0.88	40.3±7.1	0.57	25.0±6.8	0.37	35.1±11.7	0.00
26-50%	51.1±9.5		40.0±8.3		29.0±9.6		40.8±9.6	
51-75%	51.9±15.2		44.4±6.0				47.8±11.2	
75-100%	55.8±10.9		42.3±4.9		33.0±13.5		47.4±13.2	
Hb (g/dL, mean±SD)								
0-25%	18.2±2.8	0.94	12.0±4.3	0.41	8.5±2.1	0.31	11.0±4.4	0.00
26-50%	18.1±3.6		12.7±2.8		9.1±2.2		13.1±3.5	
51-75%	19.0±0.6		14.1±2.4		-		16.3±3.1	
75-100%	18.1±3.1		13.9±1.6		11.4±4.9		15.8±3.9	
Platelet (×10⁹/L, mean±SD)								
0-25%	459.5±217.1	0.68	1,178.7±465.1	0.69	206.9±148.0	0.2	693.7±581.5	0.07
26-50%	365.5±231.3		1,116.2±293.6		496.7±487.5		959.2±419.0	
51-75%	538.4±301.3		968.5±469.2		0		773.0±443.7	
75-100%	403.5±207.2		1,053.9±235.6		535.3±483.4		639.1±392.2	
				T allele burden				
Organomegaly		$P^†$		$P^†$		$P^†$		$P^†$
Positive	64.4±21.4	0.84†	53.4±23.7†	0.03	36.3±38.9	0.53	50.3±30.2	0.28
Negative	66.8±27.1		35.6±24.3		23.6±35.5		42.8±30.0	

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Table 3. *(Continued from the previous page)* Associations of clinicohematologic parameters with *Janus kinase 2* (*JAK2*) V617F allele burden

T allele burden	PV (N=22)	P*	ET (N=47)	P*	PMF (N=16)	P*	Total (N=85)	P*
Thrombotic event								
Positive	72.0±11.5	0.67	44.7±21.0	0.33	48.0±37.5	0.41	48.3±23.1	0.52
Negative	65.1±26.5		37.4±27.9		27.7±37.3		44.2±32.8	
Fibrosis								
Negative	80.2±7.7	0.11	49.1±27.1	0.46	-		62.1±26.0	0.05
Positive	60.7±25.1		41.3±25.0		31.5±37.0		43.9±29.7	
Mild	58.4±29.2	0.32‡	34.5±22.4	0.10‡	34.6±44.7	0.73‡	39.8±28.5	0.14‡
Moderate	57.5±22.1		44.5±25.2		17.6±27.2		45.6±23.8	
Severe	76.7±12.3		46.9±40.1		40.9±42.7		54.2±38.7	

*P values describe differences of parameters among four groups according to V617F allele burden measured (by ANOVA); †P values describe differences of mean V617F allele burden between negative and positive groups for organomegaly, thrombotic event, and fibrosis (by independent T-test); ‡P values describe differences of V617F allele burdens among mild, moderate, and severe fibrosis groups (by ANOVA).
Abbreviations: PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; WBC, white blood cell.

lated the 0-25% or 26-50% groups. No significant differences were detected when comparing mean age, WBC count, neutrophil fraction, Hb concentration, Hct, or platelet count among the 4 allele groups for each disease subgroup. However, many of these characteristics tended to increase in severity with incremental increases in the V617F allele burden in each disease subgroup. In contrast, platelet counts tended to be decreased with high V617F allele burdens. ET patients with organomegaly were significantly more likely to harbor high V617F allele burdens (53.4%±23.7%) than patients carrying wild-type *JAK2* (35.6%±24.3%) (P=0.03). Organomegaly and V617F allele burden were uncorrelated among PV and PMF patient subgroups. Patients with a history of thrombotic events displayed a slight tendency toward carrying a higher T allele burden compared with patients who did not experience thrombotic events in each disease group. The V617F allele burdens of PV and ET patients with fibrosis tended to be lower than those of patients without fibrosis, but this difference was not significant. When V617F allele burdens were stratified by the extent of fibrosis (i.e., mild, moderate, or severe) in each disease subgroup, no significant associations were observed.

When patients were stratified into 2 groups on the basis of V617F allele burden, that is, <50% and ≥50%, no significant differences were detected with regard to most of the clinicohistological parameters, except older age of PMF patients in the ≥ 50% V617F allele burden group (P=0.03).

DISCUSSION

The *JAK2* V617F acquired somatic mutation is the most com-

monly described mutation associated with MPN. Current estimates suggest that the *JAK2* V617F mutation is detected in >95% of patients diagnosed with PV, approximately 60% of ET patients, and 50% of PMF patients [19-21]. In agreement with these reports, we detected the *JAK2* V617F mutation in 95.5% of PV patients and 52.9% of PMF patients. However, ET patients in our study carried slightly higher V617F allele frequencies (68.8%) than previously reported. This might be explained by the smaller population examined in our study compared with previous study populations.

In numerous studies, the *JAK2* V617F allele has been variably associated with higher indices of erythropoiesis, unchanged or decreased platelet counts, greater occurrence of thrombosis, increased BM fibrosis or cytoreductive treatments, older age, longer disease duration, or poorer survival in MPN [2-8]. Similarly, it was reported that the *JAK2* V617F mutation in ET or PMF patients may skew their presenting phenotype toward a more erythremic and less thrombocythemic phenotype related to high erythropoietic and granulopoietic parameters and low platelet counts [4, 7]. Among ET patients in our study, we observed correlations between *JAK2* V617F positivity and older age, higher neutrophil fraction, higher frequency of thrombotic events, and higher myelofibrosis rates; these results are consistent with previous reports. However, we did not observe low platelet counts in *JAK2* V617F-positive patients. Instead, we observed a slight tendency in groups of high *JAK2* V617F allele burden to exhibit lower platelet counts among ET patients.

Similar to the *JAK2* V617F mutational status, previous studies have frequently correlated *JAK2* V617F allele burdens with features of stimulated erythropoiesis (e.g., elevated Hct and

decreased mean cell volume, serum ferritin level, and erythropoietin level), myelopoiesis (e.g., increased WBC count, neutrophil count, and serum lactate dehydrogenase level), neutrophil activation (e.g., elevated leukocyte count and alkaline phosphatase level), and high complication rate in MPN [9-18]. Compared to *JAK2* V617F heterozygous patients, *JAK2* V617F homozygous patients (>50% *JAK2* V617F allele burden) with PV or ET exhibited significantly higher Hb levels, WBC counts, and Hct; older age; larger spleen volumes; higher incidence of pruritus; and/or more frequent progression to secondary myelofibrosis [11, 12]. In another study, patients with >75% *JAK2* V617F allele burdens were at a higher relative risk of presenting with large spleens, pruritus, major cardiovascular events, or requiring chemotherapy compared with patients with a mutant allele burden <25%; thus, >75% *JAK2* V617F allele burden at diagnosis was suggested as one of the high risk factors [10]. A separate study also correlated the *JAK2* V617F allele burden with advanced myelofibrosis and greater splenomegaly [13].

The *JAK2* V617F allele burden has been associated with disease duration in PV patients, such that the allele burden was significantly elevated in patients examined more than 5 years after their initial diagnosis [14]. To minimize this potential bias, we limited our analyses to samples collected at each patient's initial diagnosis. However, we detected an association only between high V617F allele burden and a higher frequency of organomegaly in ET patients, but not for other clinicohematologic parameters among PV or PMF patients. PV, ET, and PMF patients with thrombotic events tended to have higher allele burdens, but this finding was not statistically significant.

Interestingly, a low *JAK2* V617F allele burden at diagnosis has been proposed as a surrogate marker for shortened survival in PMF patients [17]. In that study, the group with low V617F allele burden showed a significantly lower survival rate than the group with high V617F allele burden, but the *JAK2* V617F mutational status did not affect the survival rate [17]. In contrast, another study demonstrated that the *JAK2* V617F mutation was associated with poorer survival in PMF patients [18]. Although our short observation period precluded survival studies, previous findings support the likelihood of the *JAK2* V617F allele burden as a prognostic marker in PMF patients.

Our study was limited by the small patient population, particularly for the PV and PMF patient subgroups, and by our method of *JAK2* V617F allele detection. We identified *JAK2* V617F from whole BM cells, whereas most previous studies have used peripheral blood granulocytes or CD34+ cells. Since *JAK2* V617F is a myeloid lineage-specific mutation that is not found in lymphocytes, our analysis may have been influenced by a low detection rate [22, 23].

Although we could observe only a few associations of the *JAK2* V617F allele with MPN phenotype parameters, our results support the hypothesis that the mutational status or allele burden of *JAK2* V617F affects the severity of MPN-associated clinical and hematologic phenotypes with a larger allele burden, tending to produce a more severe phenotype (e.g., high myelopoiesis or high frequency of complications). Considering the limited patient number in the present study, a larger data set might improve the analysis of significance of *JAK2* V617F alleles across clinical or phenotypic subgroups of MPN patients.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgement

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