RESEARCH ARTICLE

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The *Janus kinase 2 (JAK2)* Mutation Burden is Related to Hematological Outcomes in Thai Patients with Myeloproliferative Neoplasms

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Abstract

Background: At present, data on the *Janus kinase 2 (JAK2)* mutation allele burden in Thai patients are limited. The aim of this study was to determine the prevalence of JAK2 V617F mutations and their association with the clinicohematological parameters of Thai patients with myeloproliferative neoplasms (MPNs). **Methods:** *JAK2* V617F mutation status and allele burden were analyzed in 394 MPN patients using TaqMan polymerase chain reaction (PCR) probes and ARMS technology-based technique using the Ipsogen *JAK2* RGQ PCR Kit. **Results:** The *JAK2* V617F mutation was detected in 130 of 394 patients (32.9%). Subtype-specific *JAK2* V617F detection rates were 48.1% for essential thrombocythemia (ET), 24.5% for polycythemia vera (PV), 22.2% for primary myelofibrosis (PMF) and 42.8% for Unspecified MPN patients. *JAK2* V617F-positive PV and ET subtypes had a significantly higher mean age than *JAK2* V617F-negative patients ($p = 0.0002$ and $p = 0.029$, respectively). The platelet (Plt) counts of *JAK2* V617F-positive PV, Unspecified MPN and All groups were significantly higher than those in *JAK2* V617F-negative patients ($p < 0.0001$, $p = 0.0006$ and $p < 0.0001$, respectively). The PMF subgroup had the highest mean *JAK2* V617F allele burden (52.43 ± 34.74) compared with Unspecified MPN (51.81 ± 25.63), PV (47.38 ± 28.78) and ET patients (21.12 ± 16.34) ($p < 0.0001$). According to *JAK2* V617F allele burden subtypes, only PV patients showed a significant increase in the white blood cell (WBC) count and hematocrit (Hct) related to incremental increases in the V617F allele burden ($p < 0.0001$). **Conclusions:** The allele burden of *JAK2* V617F was significantly lower in ET than in PV and PMF. Only the WBC, Hct and Plt counts were significantly different among the *JAK2* V617F allele burden subgroups. Measuring the *JAK2* V617F allele burden quantitatively might be an additional tool for predicting the hematological outcomes of MPNs.

Keywords: *JAK2* mutation- myeloproliferative neoplasms- allele burden

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Introduction

Myeloproliferative neoplasms (MPNs) are a group of hematological disorders characterized by the clonal proliferation of myeloid cells. The Janus kinase 2 (*JAK2*) mutation, particularly of the *JAK2* V617F allele, has emerged as a critical determinant in the classification and prognosis of MPNs [1]. Studies have reported that the *JAK2* V617F mutation, a common somatic mutation in MPNs, is present in varying proportions of patients with conditions including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [2, 3]. The prevalence of the *JAK2* V617F mutation has been reported to be as high as 96% in PV patients and approximately 55% to 65% in ET and PMF patients, underscoring its significance in the pathogenesis of these disorders. Moreover, investigations into the prevalence of *JAK2* mutations in different populations have revealed differing frequencies across ethnic groups. Studies of Chinese patients with chronic myeloproliferative disorders reported that the prevalence of *JAK2* mutations was 100% in PV, 62.4% in ET and 66.7% in PMF patients [2]. Similarly, research in Asian patients with ET has aimed to determine the prevalence of the mutated *JAK2* gene and its impact on disease profiles [4].

Studies have shown that *JAK2*-mutated secondary myelofibrosis is associated with an extremely high mutant allele burden, indicating a potential link between allele burden and disease transformation [5]. Furthermore, the allele burden of *JAK2* V617F has been correlated with hematologic parameters, clinical findings and prognosis in MPNs [6, 7] and is implicated in the risk of transformation to myelofibrosis, further emphasizing its significance in disease progression [8]. The allele burden of *JAK2* V617F

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was shown to influence the predisposition to specific MPN subtypes, differential activation of signal transducer and activator of transcription, and the order of other somatic mutations [9], as well as treatment responses and the differential diagnosis of Philadelphia chromosome-negative MPNs [10, 11]. Furthermore, the *JAK2* mutation burden has been associated with genetic instability and the transition to *JAK2* V617F homozygosity, which is critical for predicting the evolution of MPNs [12]. Importantly, the allele burden of *JAK2* V617F affected the disease susceptibility and survival of patients with primary myelofibrosis, independent of mutational status [13]. Additionally, the presence and allele burden of *JAK2* V617F have been investigated in the context of allogeneic stem cell transplantation for myelofibrosis, highlighting its impact on disease clearance and outcomes [14]. Studies on the impact of *JAK2* mutations on treatment responses and disease monitoring have evaluated the reduction of the *JAK2* allele burden in response to specific therapies, such as ruxolitinib, in patients with PV, highlighting the potential clinical benefits of allele burden reduction [15]. Monitoring the *JAK2* mutation burden after treatment has been proposed as a valuable tool for assessing treatment efficacy and disease progression in MPN patients [16]. At present, data on the Jak2 mutation allele burden in Thai patients are limited. In this study, we performed quantitative real-time polymerase chain reaction (PCR) to determine the mutational status and mutated allele burden of *JAK2* in 588 Thai patients. Additionally, we conducted an analysis of how the burden of the *JAK2* V617F mutant allele influences disease phenotypes, as well as clinical and hematological characteristics.

Materials and Methods

Patients and Samples

Blood and bone marrow samples from suspected MPN patients (n = 394) defined by clinicians from various hospitals or organizations in Thailand were analyzed by the Molecular and Genomic Research Laboratory, Chulabhorn Learning and Research Centre, Chulabhorn Royal Academy for *JAK2* V617F from January 2019 to December 2023. The Ethics Committee of Human Research of Chulabhorn Research Institute approved this study (EC No. 059/2565). Total genomic DNA was isolated from blood samples following the protocol of the QIAmp® Blood mini kit (QIAGEN, Maryland, USA). Genomic DNA from bone marrow samples was isolated using the DNAZOL® Reagent (Life Technologies, MA, USA) following the manufacturer's protocol. Isolated DNA was then quantified and qualified by A260/280 measurements using a NanoDrop One Instrument (Thermo Scientific, Massachusetts, USA).

JAK2 Allele Burden Analysis

To measure the Jak2 allele burden, the ipsogen *JAK2* RGQ PCR Kit (QIAGEN, Maryland, USA) was used. This analysis method uses TaqMan PCR probes and amplification refractory mutation system (ARMS) technology on a Rotor-Gene Q MDx instrument (QIAGEN, Maryland, USA) for the precise detection of the *JAK2*V617F mutation. The results are based on a threshold of a minimum of 1% mutant allele frequency. The kit reports the percentage of the *JAK2* V617F mutant allele relative to the total *JAK2* wildtype using quantitation curves. To ensure accuracy, the kit includes positive and negative controls for wildtype and mutant samples in every run, as well as an internal control in a separate fluorescence channel for each sample. The procedures of RQ-PCR are in brief as followed; preparation of master mix that composed of *JAK2* WT Reaction Mix, Taq DNA polymerase and DNA samples into 0.1 mL strip tube with total volume of 25 µl. Mix gently by pipetting up and down then load to the Rotor-Gene Q instrument. After that, program the Rotor-Gene Q instrument with the thermal cycling program as followed; Mode of analysis is quantitation. Hold Temperature is 95°C for 10 min. For cycling step composed of 45 cycles with 95°C for 15 sec., 60°C for 1 min with acquisition of FAMTM fluorescence in channel Green is single and acquisition of HEX fluorescence in channel Yellow as single. After that start the step of Auto-Gain Optimization settings and thermal cycling program as manufacturing protocol. For the interpretation of results, the data for the threshold cycle (CT) values can be exported from the qPCR instrument and pasted into an Excel® file for analysis.

Statistical Analysis

Associations between mutations and demographic data were analyzed by parametric (independent t-test and ANOVA) or non-parametric (Mann–Whitney and Wilcoxon) tests. Statistical analyses were performed using GraphPad Prism 10.0 (GraphPad Software, Inc., San Diego, CA, USA).

Results

The *JAK2* allele burden was determined in 394 blood and bone marrow samples from the patients. The samples were classified into four subtypes for further analysis, including PV, ET, PMF and Unspecified MPN. The classification of MPN patients is according to the 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms and Guidelines for the Diagnosis and Management of MYELOPROLIFERATIVE NEOPLASMS (MPN) 2023 from The Thai Society of Hematology. However, in term of unspecified MPN patients were defined by clinicians from the other hospitals outside Chulabhorn Royal Academy and might be not classified into subtype of MPNs according to the guideline that mentioned above. Table 1 and supplementary Table 1 present the number and hematologic characteristics of the samples of each subtype. The mean age of each subtype is as follows: PV (53.00 ± 14.56 years), ET (57.33 ± 16.92 years), PMF (65.31 ± 9.66 years) and Unspecified MPN (54.95 ± 17.38 years). The mean age of patients with PMF was significantly higher than that of the ET and PV patients ($p < 0.05$ and $p < 0.0001$, respectively). The mean white blood cell (WBC) counts of the PV group were significantly lower than those of the ET and PMF groups ($p < 0.05$ and $p < 0.0001$, respectively). The mean hematocrit (Hct) and hemoglobin (Hb) concentrations

Table 1. Hematologic Characteristics According to Myeloproliferative Neoplasm Subtype

Parameters	PV	ET	PMF	Unspecified MPN	Total
Number of patients (male: female)	208 (170:38)	129 (55:74)	36 (20:16)	21 (12:9)	394 (257:137)
Age (year, mean ± SD)	53.00 ± 14.56	57.33 ± 16.92	65.31 ± 9.66	54.95 ± 17.38	55.64 ± 15.55
WBC (10 × 9/L, mean ± SD)	10.62 ± 5.93	15.38 ± 16.85	23.29 ± 21.9	16.97 ± 17.24	13.80 ± 13.56
Hb (g/dL, mean ± SD)	17.46 ± 2.37	11.84 ± 2.18	10.40 ± 9.89	11.76 ± 4.25	14.69 ± 4.69
Hct (%, mean ± SD)	53.53 ± 7.31	36.76 ± 6.49	27.07 ± 7.98	37.32 ± 13.60	44.93 ± 12.19
Plt (10 × 9/L, mean ± SD)	340.8 ± 284.1	904.3 ± 467.5	320.8 ± 331.2	701.6 ± 1,097.73	547.2 ± 507.8
Residue of JAK2-V617F (%, mean ± SD)	47.38 ± 28.78	21.12 ± 16.34	52.43 ± 34.74	51.81 ± 25.63	35.47 ± 27.29
N of *JAK2* V617F-positive (%)	51 (24.5)	62 (48.1)	8 (22.2)	9 (42.8)	130 (32.9)

PV, polycythemia vera; ET, essential thrombocythemia; Plt, platelet; PMF, primary myelofibrosis; MPN, myeloproliferative neoplasm; WBC, white blood cells; Hct, hematocrit; Hb, hemoglobin; SD, standard deviation.

were significantly different by disease subgroup, with PV patients exhibiting the highest values, followed by ET and PMF patients, in that order ($p < 0.001$). The Unspecified MPN subtypes had significantly lower Hct concentrations than the PV and PMF subtypes ($p < 0.01$ and $p < 0.05$, respectively). The hematologic parameters of the Unspecified MPN group were similar to those of the ET group. The mean platelet (Plt) counts were highest in ET patients ($p < 0.001$; compared with the PV and PMF groups) and did not differ significantly between PV and PMF patients. The *JAK2* V617F mutation was detected in 130 of 394 patients (32.9%). Subtype-specific *JAK2* V617F detection rates were 48.1% for ET, 24.5% for PV, 22.2% for PMF and 42.8% for Unspecified MPN patients. Table 2 presents a comparison of hematologic data from *JAK2* V617F-positive and *JAK2* V617F-negative patients in the disease subgroups. No significant differences were detected in the WBC, Hb and Hct between the disease subgroups. In contrast, the *JAK2* V617F-positive PV and ET subtypes had a significantly higher mean age than the *JAK2* V617F-negative patients ($p = 0.0002$ and $p = 0.029$, respectively). Moreover, the Plt counts in *JAK2* V617F-positive PV, Unspecified MPN and All groups were significantly higher than those in *JAK2* V617F-negative patients ($p < 0.0001$, $p = 0.0006$ and $p < 0.0001$, respectively).

JAK2 V617F allele burdens were measured for 130 of the 394 patients in the study population. This corresponded to 51 PV patients, 62 ET patients, 8 PMF patients and 9 Unspecified MPN patients (Table 1). The PMF subgroup had the highest mean *JAK2* V617F allele burden (52.43 ± 34.74) compared with Unspecified MPN (51.81 ± 25.63), PV (47.38 ± 28.78) and ET patients (21.12 ± 16.34) ($p < 0.0001$). To examine the relationship between hematologic parameters and *JAK2* V617F allele burden, PV, ET and PMF patients were stratified by *JAK2* V617F allele burden as follows: < 25%, 26%–50%, 51%–75%, or 76%–100% (Table 3). The patients were most frequently classified into the < 25% allele group, and this was only significantly different compared with the ET group ($p < 0.0001$). No significant differences were detected when comparing the mean age and Hb concentration among the four allele groups for each disease subgroup. Moreover, many characteristics tended to increase in severity, with incremental increases in the *JAK2* V617F allele burden in each disease subgroup. PV patients especially had a significant increase in WBC counts and Hct related to incremental increases in the *JAK2* V617F allele burden ($p < 0.0001$). When hematologic parameters were compared across all *JAK2* V617F-positive patients stratified by allele burden subtype, the WBC, Hb and Hct were significantly different between each allele group ($p = 0.072$, $p = 0.022$ and $p = 0.0007$, respectively).

Discussion

The *JAK2* V617F mutation is a critical genetic alteration associated with MPNs, and its prevalence varies significantly across different geographical regions and populations. This mutation is particularly prevalent in patients with PV, where studies have reported frequencies exceeding 90% in various cohorts [17, 18]. In contrast, the prevalence of the *JAK2* V617F mutation in ET and PMF is notably lower, with estimates ranging from 50% to 60% [18]. We analyzed 394 samples for *JAK2* V617F status and allele burden using a commercially available *JAK2* RGQ PCR kit that uses TaqMan PCR probes and ARMS technology. Of the 394 samples, 130 (32.9%) were confirmed as positive with the highest mutation frequency in the ET group (48.1%), followed by Unspecified MPN (42.8%), PV (24.5%) and PMF (22.2%). Our data showed a lower mutation frequency in our population compared with previous reports. The difference in our data compared with previous studies may be related to our samples being collected from abnormal myeloid proliferative patients with no data to confirm them as Philadelphia (Ph) negative, whereas most previous reports analyzed in Ph negative patients [10, 19, 20]. Geographical differences also play a significant role in the prevalence of the *JAK2* V617F mutation. For example, a study of Korean patients indicated a higher frequency of *JAK2* exon 12 mutations compared with Western populations, suggesting ethnic variations in mutation profiles [21]. Similarly, Chinese patients with PV had a lower frequency of the *JAK2* V617F mutation (approximately 82%), but a higher prevalence of exon 12 mutations (around 13%) than typically observed in Western cohorts [22]. These findings highlight the importance of considering ethnic and regional factors when assessing the prevalence of *JAK2* mutations. According to disease subtypes, we found the significance differences of hematological parameters including Hb, Hct, WBC and Platelet counts. We found

Table 2. Hematologic Characteristics According to *Janus kinase 2* (*JAK2*) Mutation Status

Parameters	PV			ET			PMF			Unspecified MPN			All groups		
	JAK2 V617F-positive	*JAK2* V617F-negative	p value	*JAK2* V617F-positive	*JAK2* V617F-negative	p value	*JAK2* V617F-positive	*JAK2* V617F-negative	p value	*JAK2* V617F-positive	*JAK2* V617F-negative	p value	*JAK2* V617F-positive	*JAK2* V617F-negative	p value
N of patients (%)	51 (24.5)	157 (75.5)		62 (48.1)	67 (51.9)		8 (22.2)	28 (77.8)		9 (42.8)	12 (57.2)		130 (33.0)	264 (67.0)	
Male: Female	30:21:00	140:17:00		28:34:00	27:40:00		5:03	15:13		4:05	8:04		67:63	190:74	
Age (year, mean ± SD)	61.10 ± 11.47	50.36 ± 14.51	0.0002	61.60 ± 16.43	53.39 ± 16.52	0.029	65.95 ± 9.0	65.18 ± 9.99	ns	67.44 ± 12.13	45.58 ± 14.80	0.015	62.06 ± 13.98	52.48 ± 15.33	< 0.0001
WBC (10 × 9/L, mean ± SD)	15.14 ± 7.52	9.09 ± 4.35	ns	17.26 ± 21.0	13.59 ± 11.50	ns	34.85 ± 32.89	19.91 ± 17.05	ns	29.41 ± 21.48	12.37 ± 8.32	0.048	12.36 ± 13.23	18.69 ± 18.29	0.0001
Hb (g/dL, mean ± SD)	17.64 ± 2.61	17.41 ± 2.28	ns	12.31 ± 2.02	11.40 ± 2.25	ns	9.28 ± 2.07	10.68 ± 11.04	ns	12.19 ± 3.87	11.44 ± 4.65	ns	14.26 ± 3.70	14.91 ± 5.11	ns
Hct (%, mean ± SD)	56.12 ± 7.61	52.67 ± 7.02	ns	38.28 ± 6.09	35.23 ± 6.58	ns	29.54 ± 6.77	26.35 ± 8.29	ns	41.23 ± 13.14	35.04 ± 13.9	ns	45.06 ± 11.74	44.86 ± 12.43	ns
Plt (10 × 9/L, mean ± SD)	617.6 ± 357.8	246.0 ± 172.7	< 0.0001	920.2 ± 491.5	888.9 ± 446.6	ns	315.9 ± 283.3	322.2 ± 349.4	ns	1,136.49 ± 1513.07	345.8 ± 385.5	0.0006	783.3 ± 597.8	424.3 ± 403.7	< 0.0001
Residue of *JAK2*-V617F (%, mean ± SD)*	47.38 ± 28.78	NA		21.12 ± 16.34	NA		52.43 ± 34.74	NA		51.81 ± 25.63	NA		36.85 ± 28.06	NA	

PV, polycythemia vera; ET, essential thrombocythemia; Plt, platelet; PMF, primary myelofibrosis; MPN, myeloproliferative neoplasm; WBC, white blood cells; Hct, hematocrit; Hb, hemoglobin; SD, standard deviation. *One-way ANOVA compared PV, ET, PMF and Unspecified MPN, $p < 0.0001$.

Table 3. Hematologic Characteristics According to *Janus kinase 2 (JAK2)* Allele Burden

Group of allele burden	PV (N = 51)	p value	ET (N = 62)	p value	PMF (N = 8)	p value	Unspecified MPN (N = 9)	p value	Total (N = 130)	p value
N of patients (%)										
< 25%	15 (29.4%)		43 (69.4%)	< 0.0001*	2 (25%)		1 (11.1%)		61 (46.9%)	
26–50%	13 (25.5%)		15 (24.2%)		2 (25%)		4 (44.4%)		34 (26.2%)	
51–75%	12 (23.5%)		3 (4.8%)		1 (12.5%)		1 (11.1%)		17 (13.1%)	
76–100%	11 (21.6%)		1 (1.6%)		3 (37.5%)		3 (33.4%)		18 (13.8%)	
Age (year, mean ±SD)										
< 25%	55.07 ± 9.93	ns	60 ± 17.69	ns	59.5 ± 9.19	n/a	58	ns	58.74 ± 15.75	ns
26–50%	62.08 ±1 3.65		65.87 ± 13.66		64.90 ± 9.90		73.75 ± 8.88		65.24 ± 13.04	
51–75%	64.83 ± 9.84		59.33 ± 9.61		75		66		64.53 ± 9.50	
76–100%	64.09 ± 10.34		73		68 ± 10		62.67 ± 17.2		65.00 ± 10.86	
WBC (10 × 9/L, mean ± SD)										
< 25%	9.32 ± 4.43	< 0.0001	17.11 ± 24.23	ns	52.54 ± 61.91	n/a	12.8	ns	16.29 ± 23.16	0.0272
26–50%	13.04 ± 3.22		17.21 ± 8.27		6.1		25.93 ± 7.44		16.23 ± 7.60	
51–75%	16.66 ± 4.32		14.74 ± 12.26		14.18		17.4		16.16 ± 5.87	
76–100%	24.17 ± 8.13		32		39.54 ± 18.85		43.62 ± 35		30.41 ± 17.19	
Hb (g/dL, mean ± SD)										
< 25%	16.31 ± 2.35	ns	12.39 ± 2.11	ns	9.45 ± 0.07	n/a	13.7	ns	13.28 ± 2.78	0.022
26–50%	18.83 ± 1.98		12.67 ± 1.63		N/A		14.88 ± 3.41		15.63 ± 3.54	
51–75%	18.04 ± 3.71		9.33 ± 1.0		5.3		12.5		15.20 ± 5.31	
76–100%	17.69 ± 1.82		11		10.5 ± 0.69		8 ± 0.75		14.51 ± 4.43	
Hct (%, mean ± SD)										
< 25%	49.11 ± 6.52	< 0.0001	38.3 ± 6.08	ns	30.05 ± 1.20	n/a	41.5	ns	40.79 ± 7.85	0.0007
26–50%	59.28 ± 6.46		39.85 ± 6.16		24		51.40 ± 11.9		48.60 ± 12.19	
51–75%	61.45 ± 4.37		33.33 ± 0.57		17.5		40		51.47 ± 15.68	
76–100%	57.1 ± 5.92		30		35.07 ± 1.65		26.45 ± 1.91		48.01 ± 13.75	
Group of allele burden	PV (N = 51)	p value	ET (N = 62)	p value	PMF (N = 8)	p value	Unspecified MPN (N = 9)	p value	Total (N = 130)	p value
Plt (10 × 9/L, mean ± SD)										
< 25%	428.5 ± 232.6	0.0446	971.2 ± 472.7	ns	95.5 ± 81.32	n/a	998	ns	809.5 ± 491.7	ns
26–50%	656.6 ± 304		876.7 ± 563.4		230		667.6 ± 468.2		736.60 ± 454.2	
51–75%	629.9 ± 238.8		500.7 ± 361.1		4		1197		600.10 ± 329.9	
76–100%	817.9 ± 526		551		595.3 ± 139.2		1,788 ± 2782		927.60 ± 1114	

PV, polycythemia vera; ET, essential thrombocythemia; Plt, platelet; PMF, primary myelofibrosis; MPN, myeloproliferative neoplasm; WBC, white blood cells; Hct, hematocrit; Hb, hemoglobin; SD, standard deviation; N, number. *Fisher's exact test was used to compare the frequency of data between PV and ET and one-way ANOVA was used to compare all subtypes.

the mean white blood cell (WBC) counts of the PV group were significantly lower than ET group ($p < 0.05$). However, in general, patients with PV tend to have higher WBC counts compared to those with ET, which is a critical distinction in the clinical management of these conditions [23-25]. The controversy of this result may be from the treatment of hydroxyurea which has previously reported the significant therapeutic effects to decrease white blood cell (WBC) counts in patients with PV, which is crucial for managing the disease and reducing the risk of thrombotic events associated with elevated blood cell counts [26, 27].

In this study, the mean *JAK2* V617F allele burden in the entire population of patients was 36.85%, ranging from 0.04% to 100%. The mean *JAK2* V617F allele burden in PMF patients was the highest at 52.43%, followed by unspecified MPN patients at 51.81%, PV patients at 47.38% and ET patients with the lowest mean *JAK2* V617F allele burden at 21.12%. One study reported that the median *JAK2* V617F allele burden in the entire population of patients was 73%, ranging from 0.97% to 97%. The median *JAK2* V617F allele burden in PV patients was 40% and in MF it was 95%. Furthermore, all PMF patients harbored more than 80% *JAK2* V617F cells, whereas ET patients had fewer than 50% *JAK2* V617F cells [28]. Other studies have reported that patients with ET are seldom homozygous for the *JAK2* V617F mutation. As a result, the allele burden of the *JAK2* V617F mutation in ET patients is generally lower compared with that observed in patients with PV and PMF [29-33]. Sang Hyuk Park et al. also reported that the allele burden of *JAK2* V617F in ET was significantly lower than that in PV and PM [10]. In addition, the *JAK2* V617F allele was shown to have variable associations with increased markers of erythropoiesis, stable or reduced Plt counts, a higher likelihood of thrombosis, more frequent bone marrow fibrosis or cytoreductive treatments, older age, longer disease duration or poorer survival outcomes in those with MPNs [34-40]. Our results were similar to previous reports in that the clinicohematological outcomes differed significantly among the disease subtypes based on the higher mean age and WBC counts for PMF than for the other subtypes, higher Hb and Hct for PV and higher Plt counts for ET than for the other subtypes (Tables 1 and supplementary table 1). A previous study reported that among ET patients, *JAK2* V617F positivity was associated with older age, a higher neutrophil fraction, an increased frequency of thrombotic events and elevated rates of myelofibrosis. However, they did not find low Plt counts among *JAK2* V617F-positive patients. Instead, they noted a slight trend in which groups with a higher *JAK2* V617F allele burden had lower Plt counts [19].

There were significant differences between *JAK2* V617F-positive and *JAK2* V617F-negative groups among all MPN subtypes in this study, including older age, increased Plt counts, and lower WBC counts in *JAK2* V617F-positive cases. Moreover, a significantly higher mean age was present in the *JAK2* V617F-positive sample PV and ET subgroups. In addition, *JAK2* V617F-positive PV patients had significantly increased Plt counts compared with the negative group (Table 2).

When this study categorized the *JAK2* V617F allele burden in the four subgroups (<25%, 26%–50%, 51%–75% and 76%–100%) and analyzed the correlation with clinicohematological parameters, we found significant differences in the PV subgroup only. An increase in the *JAK2* V617F allele burden tended to be associated with an increase in the WBC, Hct and Plt counts ($p < 0.0001$, $p < 0.0001$ and $p < 0.05$, respectively). These results differed from a previous study that found an association between a high *JAK2* V617F allele burden and an increased frequency of organomegaly in ET patients without identifying similar links for other clinicohematological parameters in PV or PMF patients. Although patients with PV, ET and PMF who experienced thrombotic events generally exhibited higher allele burdens, this association was not statistically significant [19]. The discrepancy in the data may be related to the lower sample size used for analysis compared with our study.

Other studies reported that individuals harboring the *JAK2* V617F mutation, regardless of MPN type, are at a higher risk of venous thromboembolism (VTE). In addition, a *JAK2* allele burden higher than 20% identified patients with a 7.4-fold increased risk of VTE [20].

The *JAK2* V617F mutation serves as a critical marker for the pathogenesis of MPNs, particularly in BCR/ABL-negative cases, where it is often the primary driver of the disease [41, 42]. In the context of treatment, the presence of the *JAK2* V617F mutation has implications for therapeutic strategies, particularly with the advent of *JAK2* inhibitors that target the JAK/STAT signaling pathway [38]. Detection of the mutation not only aids in the diagnosis of MPNs but also assists in monitoring disease progression and treatment responses [6, 43, 44].

Our study was limited by the small number of parameters used for comparisons according to the samples were sent from various hospitals or organizations with no other additional information including, treatment regimen, treatment outcomes and disease progression. However, we observed many associations between *JAK2* V617F status and allele burden with clinicohematological parameters. Our findings confirm that the mutational status or allele burden of *JAK2* V617F influences the severity of the clinical and hematologic symptoms associated with MPNs. Specifically, a higher allele burden is generally associated with more severe manifestations, such as elevated WBC or Plt counts.

In conclusion, our study findings show that the allele burden of *JAK2* V617F was significantly lower for ET than for PV and PMF. ET patients also had a significantly lower frequency of the *JAK2* V617F allele, whereas the other MPN subgroups had a similar frequency. Only the WBC, Hct and Plt counts were significantly different between the *JAK2* V617F allele burden subgroups. Measuring the *JAK2* V617F allele burden quantitatively might be an additional tool for predicting the hematological outcomes of the disease. This approach will require the assessment of the *JAK2* V617F allele burden at the time of diagnosis and during ongoing patient monitoring. According to the limited parameters in this study, expanding the dataset and including additional clinical parameters might provide a more comprehensive analysis of the significance of *JAK2* V617F alleles across various clinical and phenotypic

subgroups of MPN patients.

Author Contribution Statement

The authors confirm contribution to the paper as follows: Miss Nattida Cholnakasem, Mr. Nithiphut Tantirukdham, Mr. Kriangpol Wiriyaukaradecha; help with sample processing and gene mutation analysis: Miss Maneenop Yimyaem and Miss Natcha Aksoramat; Data collection: The authors thank Dr.Narongrit Sritana; statistical analysis and advised in reviewing the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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Ethical Declaration

This study was approved by the Ethics Committee of Human Research of Chulabhorn Research Institute (EC No.059/2565).

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