Original Article

Prevalence of JAK2V617F Mutation in 1,247 Thai Patients with Suspected Myeloproliferative Neoplasms

Chakrit Bunyoo MSc[1], Kriangpol Wiriyaukaradecha MSc[1],
Narongrit Sritana MSc[1], Wandee Udomchaiprasertkul MSc[1],
Nithiphut Tantirukdham BSc[1], Jin Tongsom MSc[1], Chirayu Auewarakul MD, PhD[2,3]

[1] Molecular Biology and Genomic Research Laboratory, Division of Research and International Relations, HRH Princess Chulabhorn College of Medical Science, Chulabhorn Royal Academy, Bangkok, Thailand
[2] Department of Medicine, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand
[3] Faculty of Medicine and Public Health, HRH Princess Chulabhorn College of Medical Science, Chulabhorn Royal Academy, Bangkok, Thailand

Objective: To determine the frequency of JAK2V617F mutation in Thai patients with clinically suspected myeloproliferative neoplasms [MPNs] including polycythemia vera [PV], essential thrombocythemia [ET], primary myelofibrosis [PMF] and unclassified MPN.

Materials and Methods: Overall, 1,247 samples were tested for JAK2V617F from January 2009 to May 2016. DNA was extracted from leukocytes separated from peripheral blood or bone marrow. JAK2V617F was detected by allele specific polymerase chain reaction [AS-PCR] followed by agarose gel electrophoresis analysis.

Results: The proportion of JAK2V617F positivity in disease subtypes was 49% for suspected PV, 54% for suspected ET, 43% for suspected PMF and 45% for unclassified MPN**.** The presence of JAK2V617F in each disease subtype was associated with a significant overproduction of the blood compartments including increased leukocyte counts (p<0.001) and platelet counts in PV (p<0.001), increased leukocyte counts (p<0.001), hemoglobin levels (p<0.001) and hematocrits in ET (p<0.001), and increased platelet counts in PMF (p = 0.023) as compared to JAK2-wild type.

Conclusion: JAK2V617F was present in approximately half of the test samples with a high clinical suspicion of various types of MPNs. The prevalence of JAK2V617F in clinically suspected PV cases was much lower than expected.

Keywords: Myeloproliferative neoplasms; JAK2V617F mutation; Allele specific PCR; Polycythemia, Thrombocythemia

J Med Assoc Thai 2018; 101 [Suppl. 6]: S157-S164
Website: http://www.jmatonline.com

Myeloproliferative neoplasms [MPNs] are a group of hematopoietic stem cell disorders that exhibit excessive clonal myeloproliferation features such as peripheral blood granulocytosis, thrombocytosis and/or erythrocytosis[1]. According to the revised 2016 World Health Organization [WHO] classification, MPNs are mainly classified into the disease sub-types: chronic myelogenous leukemia [CML], Chronic neutrophilic leukemia [CNL], polycythemia vera [PV], essential thrombocythemia [ET] and primary myelofibrosis [PMF][2]. BCR-ABL tyrosine kinase fusion has been implicated in most CML[3]. The three BCR-ABL-negative related disorders (PV, ET and PMF) involve an acquired somatic point mutation in the JAK2 gene known as JAK2V617F–a guanine to thymidine base transversion at nucleotide 1849 in exon14 of JAK2 caused by an amino acid substitution from valine (V) to phenylalanine (F) at residue 617 of the JAK2 protein. The mutation revokes JAK2 auto-inhibition resulting in the constitutive activation of JAK signaling. This dysregulation leads to excessive clonal myeloid cells in the bone marrow and peripheral blood[4-6]. Since 2008,

Correspondence to:
Auewarakul C. HRH Princess Chulabhorn College of Medical Science, Chulabhorn Royal Academy, 54 Kamphaengphet 6 Rd, Talat Bangkhen, Laksi, Bangkok 10210, Thailand.
Phone: +66-2-5766864, **Fax:** +66-2-5766392
E-mail: chirayu.aue@pccms.ac.th

How to cite this article: Bunyoo C, Wiriyaukaradecha K, Sritana N, Udomchaiprasertkul W, Tantirukdham N, Tongsom J, Auewarakul C. Prevalence of JAK2V617F Mutation in 1,247 Thai Patients with Suspected Myeloproliferative Neoplasms. J Med Assoc Thai 2018;101;Suppl.6: S157-S164.

the presence of JAK2V617F has been adopted as a major WHO-diagnostic criteria for PV, ET and PMF[7].

The prevalence of JAK2V617F in Western and Asian countries has been reported. In Western countries, the mutation is found most frequently in PV (80 to 90%), then ET (50 to 60%) and PMF patients (30 to 50%)[8]. In Asian countries, JAK2V617F was commonly found in patients with PV (77 to 100%), then ET (56 to 71%) and PMF (42 to 70%)[9-16]. However, there are limited reports of JAK2V617F prevalence in Thai MPN patients. The aims of this study were to determine the (i) prevalence of the JAK2V617F mutation and (ii) investigate a correlation between the mutation status and clinical characteristics of Thai patients with suspected MPNs, including PV, ET and PMF.

Materials and Methods

Patient samples

This study was approved by the Ethics Committee for Human Research, Chulabhorn Research Institute (EC No. 016/2554). The samples of bone marrow and/or peripheral blood from suspected MPN patients (n = 1,247) diagnosed by clinicians were sent to the Molecular Biology and Genomic Research Laboratory, HRH Princess Chulabhorn College of Medical Science (Chulabhorn Hospital) for JAK2V617F screening from January 2009 to May 2016. The total MPN patients comprised PV (n = 555), ET (n = 433), PMF (n = 70) and unclassified MPN cases (n = 189).

Genomic DNA extraction

Leukocytes were separated from samples by lysis of erythrocytes and centrifugation. Bone marrow or blood was transferred into a sterile 15 mL tube containing 10 mL RBC buffer (155 mM NH_4Cl, 12 mM $NaHCO_3$, 0.1 mM EDTA, pH 8) and incubated for 10 minutes (min) at room temperature on rotating platform. The suspension was centrifuged at 2,500 g for 10 min and the supernatant was removed without disturbing the pellet. If erythrocytes were still present; 15 mL RBC lysis buffer was added to the pellet, then incubated and centrifuged as previous step. After the supernatant was removed, 15 mL phosphate buffer saline [PBS] was added to the pellet and mixed by inverting. The suspension was centrifuged at 2,500 g for 10 min and the supernatant was removed. The leukocyte pellet was transferred to a sterile 1.5 mL tube and extracted genomic DNA using DNA zolreagent (Invitrogen, USA) in accordance with the manufacturer's instructions. The isolated DNA was quantified and qualified by A260/A280 measurement performing on Nanodrop ND-1000 (NanoDrop Technologies, USA) instrument.

Allele-specific PCR [AS-PCR] for JAK2V617F

AS-PCR was performed using a common primer (R) (5′-CTGAATAGTCCTACAGTGTTTTCAG TTTCA-3′), a forward mutant specific primer (FM) (5′-AGCATTTGGTTTTAAAT TATGGAGTATATT-3′), and a forward internal control primer (FC) (5′-ATC TATAGTCATGCTGAAAGTAGGAGAAAG-3′), as previously described[4]. Then, 200 ng of DNA was added to a 25 µL reaction mixture containing 10 pmol R, 10 pmol FM, 0.25 pmol FC primer, 2.5 mmol/L $MgCl_2$, 0.2 mmol/L each of dNTP, and 0.625 U of Platinum Taq DNA Polymerase (Invitrogen, USA). The PCR was performed in a C-1000 thermal cycler (BioRad, USA). The PCR conditions were an initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 seconds (s), annealing at 64°C for 30 s, extension at 72°C for 30 s, and a final cycle at 72°C for 2 min. PCR products were separated with 2% agarose gel in 0.5xTris borate EDTA buffer (TBE; 40 mMTris-Cl, pH 8.3, 45 mM boric acid, 1 mM EDTA) and visualized in gel imaging systems (Syngene, USA) after staining with ethidium bromide. The mutant and internal control products were 203 bp and 364 bp in length, respectively.

Any sample which revealed an ambiguous mutant band was verified by two-round AS-PCR[17]. Then, 1 µL of PCR product from the primary amplification was re-amplified and then used for AS-PCR as described above.

Assay sensitivity determination

A mixture of DNA was derived from the human erythroleukemia cell line, HEL (Cell Lines Service, Germany) and human chronic myeloid leukemia cell line, K562 (ATCC, USA). Fifty ng/µL of DNA from HEL carrying the homozygous mutant for JAK2V617F was serially diluted with 50 ng/µL of DNA from K562 (homozygous wild-type for JAK2V617F) to prepare 9 dilutions of DNA template with the following percentages of JAK2V617F allele burden: 100%, 50%, 10%, 5%, 2.5%, 1%, 0.5%, 0.1% and 0%. AS-PCR for JAK2V617F was performed as previously described.

Statistical analysis

Patient characteristics were reported as the median and range for all continuous variables and as proportions and absolute counts for discrete variables. A comparison of clinical features between patients carrying JAK2V617F and JAK2-wild-type was

performed by Mann-Whitney U-test (non-normal distribution) or Independent t-test (normal distribution) for age, leukocyte count, hemoglobin level [Hb], platelet count and percentage of hematocrit (% Hct) and Fisher's exact test for gender. A *p*-value of <0.05 was considered statistically significant.

Results

Sensitivity of AS-PCR for JAK2V617Fdetection

The AS-PCR used previously reported primer sets providing 2 sizes of PCR products (4). Primers FC and R amplified a 364-bp PCR product from mutant and wild-type alleles, which was used as an internal control. Primers FM and R amplified a 203-bp PCR product that indicated the presence of the JAK2V617F mutation. Assay sensitivity evaluation indicated that AS-PCR in the present study clearly detected the 5% dilution. The mutant band was ambiguous in the 1% to 0.5% dilution and was completely absent in the 0.1% to 0% dilution or negative control samples (Figure 1). Any patient samples that gave a faint mutant band (carrying a very low amount of JAK2V617F allele), underwent two-round AS-PCR to confirm the result (data not shown).

Prevalence and clinical correlations of JAK2V617F in suspected MPN patients

Overall, JAK2V617F was detected in 49.7% patients (620 of 1,247) clinically suspected for MPNs by clinicians. The proportion of JAK2V617F positivity in disease subtypes was 49% for suspected PV (272/555 samples), 54% for suspected ET (232/433), 43% for suspected PMF (30/70) and 45% for unclassifiable MPN (86/89). The characteristics of enrolled patients in each disease subtype including age at diagnosis, gender and general hematological parameters are shown in Table 1.

At diagnosis, the presence of JAK2V617F in suspected PV was associated with older age ($p<0.001$), higher leukocyte counts ($p<0.001$), and platelet counts ($p<0.001$) than that of patients carrying JAK2 wild type. In suspected ET, the mutation was associated with older age ($p<0.001$), significantly higher leukocyte counts ($p<0.001$), hemoglobin level ($p<0.001$) and percentage of hematocrit ($p<0.001$). Cases with suspected PMF carrying the mutation were associated with significantly higher platelet counts ($p = 0.023$). In suspected ET and PMF, the prevalence of the mutation was similar between genders except for suspected PV where the prevalence was significantly greater in females than males ($p<0.001$) (Table 2).

Discussion

Although JAK2V617F mutation is the most well-known molecular marker for the diagnosis, prognosis and treatment of MPNs (5), its frequency and correlation with clinical data among Thai patients has not been well investigated. Our study of a large sample (n = 1,247) of suspected MPN patients focuses on the prevalence and clinical correlation of the mutation in each MPN subtype.

JAK2V617F was present in approximately half of the suspected MPN cases (46.7%). In suspected ET and PMF, the prevalence was 53% and 42%, respectively. This is comparable to global reports where the prevalence of the mutation is 50 to 60% in ET and 30 to 50% in PMF[8]. In previous Asian studies,

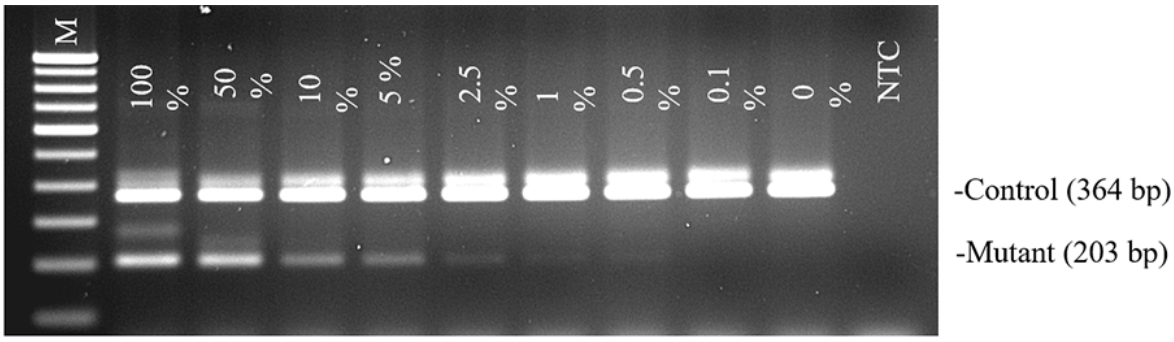

Figure 1. Assay sensitivity of AS-PCR for the JAK2V617F mutation. Nine dilutions of template DNA with 100%, 50%, 10%, 5%, 2.5%, 1%, 0.5%, 0.1% or 0%JAK2V617F allele burden were amplified byAS-PCR. The presence of the mutant allele in a sample was defined as the presence of a control band (364bp) and mutant band (203bp). The presence of the wild-type allele in a sample was defined as a control band (364bp). M = TrackIt 100 bp DNA ladder (Invitrogen, USA), NTC = no template control.

Table 1. Patient characteristics

Clinical features	Disease subtype			
	PV (n = 555)	ET (n = 433)	PMF (n = 70)	Un-classified* (n = 189)
JAK2V617F (%)	272 (49.0)	232 (53.6)	30 (42.9)	86 (45.5)
Gender (male/female)	399/156	198/235	37/33	106/83
Age (year), median (range)	56 (19 to 99)	64 (10 to 99)	65.5 (32 to 96)	63 (2 to 91)
Leukocytes, $x10^9$/L, median (range)	10.6 (0.9 to 324)	12.3 (2.0 to 500)	12.0 (<1 to 146)	13.5 (1.3 to 407.1)
Hb (g/dl), median (range)	18.2 (4.9 to 23.9)	11.6 (3.7 to 34)	8.8 (4.1 to 16.2)	11.5 (4.1 to 21.7)
Platelet, $x10^9$/L, median (range)	300 (8 to 2,871)	1,036.5 (1.7 to 7,500)	144 (2.8 to 952)	588 (1.2 to 5,000)
Hct (%), median (range)	54.6 (17 to 76.5)	35.5 (12.3 to 72)	27.7 (12.7 to 50.7)	36 (13.9 to 62.6)

* Unclassifiable MPN or uncertain diagnosis
Hb = hemoglobin; Hct = hematocrit; PV = polycythemia vera; ET = essential thrombocythemia; PMF = primary myelofibrosis

the prevalence was reported to be 56 to 71% in ET and 42 to 70% in PMF, which is in accord with our study (Table 3). This suggests that ethnicity is unlikely to affect the prevalence of the JAK2V617F mutation. However, there was a diverse frequency in the prevalence of JAK2V617F in MPNs when studying global and regional cohorts. This may be explained by the sample size effect and/or the sensitivity of each detection technique used[17-19].

Conversely, the prevalence of JAK2V617F in suspected PV in our cohort was lower (49%) compared with previous studies. The prevalence of the JAK2V617F mutation in Western or Asian cohorts was 80 to 90%[4-8] and 70 to 100% (Table 3), respectively. This indicates that PV patients in our study might be over-diagnosed by clinicians possibly because of their lower stringency or use of different diagnostic criteria rather than the sensitivity of the detection method. Regarding the sensitivity of AS-PCR used in our study, the mutant allele was detected up to 1% allele burden (Figure 1). Furthermore, any samples that showed a faint mutant band (carrying a very low amount of JAK2V617F allele), underwent two-round AS-PCR according to Kannim et al[17], which detected a mutant allele burden below 1%. These results indicate that the sensitivity of AS-PCR used in our study was clinically appropriate and sufficient despite the fact that our study investigated whole bone marrow or blood, which consisted of a mixture of myeloid, lymphoid and other stromal cells, instead of granulocyte-enriched samples.

Another reason that may explain the lower JAK2V617F prevalence in suspected PV is that these patients may carry other functionally similar mutations such as JAK2 exon12 mutation. Recently, a number of reports revealed that approximately 4% of PV patients that were JAK2V617F-negative had a mutation at exon12 of JAK2[20]. Surprisingly, a study in Taiwanese patients reported a high prevalence of JAK2 exon12 mutation (23%) in PV patients without JAK2V617F[13], which was similar to a Korean cohort in which the JAK2 exon12 mutation was present in approximately 12% of cases[21]. Besides, the prevalence of JAK2 exon12 mutation in Thai patients with PV have been found around 1%[22]. These evidences strongly support that both JAK2V617F and JAK2 exon12 mutations are exclusive diagnostic molecular markers for PV according to the 2016 revised WHO criteria[2].

The JAK2V617F mutation was significantly associated with older age, higher hemoglobin level and leukocyte counts in suspected ET ($p<0.001$) than that of JAK2-wild type. These findings are in accord with Zhou et al[23] and Kittur et al[24]. Furthermore, Speletas et al[25] reported a significant correlation between older age or higher hemoglobin level and JAK2V617F mutation but there was no significant difference in leukocyte counts in their cohort. Of note, our mutant ET and previous cohorts[23-25] displayed PV-like phenotypes (high hemoglobin level and/or high percentage of hematocrit). This suggests that mutant ET may transform and develop into true-PV[26]. In cases of suspected PV, the data suggest that mutants were significantly associated with older age, high leukocyte counts and platelet counts but low hemoglobin levels. These results are comparable to a previous Thai cohort

Table 2. Correlation of JAK2V617F mutation and clinical characteristics of patients with suspected PV, ET and PMF

Clinical features	Suspected PV			Suspected ET			Suspected PMF		
	JAK2V617F		*p*-value	JAK2V617F		*p*-value	JAK2V617F		*p*-value
	+	-		+	-		+	-	
No. patients	272	283		232	201		30	40	
Gender, M/F	147/125	252/31	<0.001	107/125	91/110	0.923	15/15	22/18	0.810
Age (year), median (range)	62 (23 to 91)	48 (19 to 99)	<0.001	69 (20 to 98)	57 (10 to 99)	<0.001	67.5 (32 to 96)	64 (39 to 87)	0.491
Leukocytes x10^9/ Lmedian (range)	17.1 (0.9 to 324)	7.7 (1.8 to 80)	<0.001	13.9 (3 to 175)	11.1 (2 to 500)	<0.001	13.4 (1.0 to 65.8)	11.4 (1.5 to 146)	0.305
Hb (g/dl), median (range)	17.9 (4.9 to 23.9)	18.4 (5.3 to 23.7)	0.004	12.1 (3.7 to 24)	10.9 (6.2 to 19.5)	<0.001	9.0 (4.1 to 16.2)	8.6 (5.0 to 13.5)	0.543
Platelet (x10^9/ L), median (range)	570 (50 to 2,871)	215 (8.0 to 1,770)	<0.001	1,050 (1.7 to 2,398)	1,020 (1.8 to 7,500)	0.610	288 (5.0 to 952)	103 (2.8 to 883)	0.010
Hct (%), median (range)	55.2 (17 to 76.5)	54.3 (17 to 73.4)	0.537	38.0 (12.3 to 72)	33.7 (20 to 59)	<0.001	28.2 (12.7 to 50.7)	27.3 (17.4 to 41)	0.399

Hb = hemoglobin; Hct = hematocrit; PV = polycythemia vera; ET = essential thrombocythemia; PMF = primary myelofibrosis

Table 3. The prevalence of JAK2V617F among Asian MPN patients in the literature

Population	Frequency of JAK2V617F [%, (n)]			Reference
	PV	ET	PMF	
Japanese	97% (66)	54% (112)	47% (23)	(10)
Korean	95% (22)	68% (64)	52% (17)	(11)
Chinese	73% (57)	58% (68)	66% (12)	(12)
Chinese	100% (35)	62% (85)	66% (3)	(13)
Chinese	NS	56% (110)	NS	(14)
Taiwanese	77% (22)	NS*	NS*	(15)
Thai	80% (31)	59% (49)	70% (10)	(16)
Thai	NS	71% (58)	NS*	(17)
Thai	49% (555)	53% (433)	42% (70)	This study

* NS = not studied in the cohort

that reported a significantly low hemoglobin level and low mean corpuscular volume of the mutant PV. This might be caused by iron deficiency or thalassemia that is frequent in Thais. These studies, however, found no correlation with age or leukocyte counts[9] whereas, Speletas et al[25] reported that the mutant PV exhibited older age and high platelet counts. Suspected PMF carrying the mutation only exhibited significantly high platelet counts. However, data for an association between the JAK2V617F mutation and the clinical characteristics of PMF reported in the literature including Thai cohorts is limited.

Significant differences in clinical characteristics between patients carrying JAK2-wild-type and JAK2V617F in our cohort indicates a critical function of JAK2 in disease progression. The JAK2V617F mutation revokes JAK2 auto-inhibition that leads to abnormal proliferation, differentiation and cell renewal. These factors might explain the overproduction of blood compartments that are the predominant phenotype of MPNs[4]. Over the last decade, investiga tions of the clinical correlations of JAK2V617F have reported varied findings. Although the reason for this divergence is unclear, JAK2V617F detection incorporated with hematologic parameters is still useful for the diagnosis, prognosis and treatment of MPNs[4].

Conclusion

We report the prevalence of JAK2V617F in a large cohort of 1,247 suspected MPNs cases including PV, ET, PMF and unclassified MPN, as studied by AS-PCR techniques. The prevalence of the mutation in ET and PMF was comparable to previous studies. Future research of altered somatic gene mutations in JAK-STAT signaling such as JAK2 exon12, MPL and CALR in JAK2V617F-negative patients should be considered.

What is already known on this topic?

The prevalence of JAK2V617F was found the most in PV and around a half of ET and PMF patients. In term of clinical characteristics, the presence of JAK2V617F leads to the overproduction of the blood compartments.

What this study adds?

The data on the prevalence of JAK2V617F mutation in MPNs was limited in Thailand. This study was performed in a large sample size (n = 1,247) of suspected Thai MPN patients. The prevalence of JAK2V617F mutation in ET and PMF was comparable to other previously reported MPNs series. However, the prevalence of JAK2V617F in clinically suspected PV cases was much lower than expected and could reflect that other causes of erythrocytosis, rather than PV, may be prevalent in Thai patients. Furthermore, altered somatic gene mutations in JAK-STAT signaling such as JAK2 exon12, MPL and CALR in JAK2V617F-negative patients should be considered.

Acknowledgements

This work was funded by Chulabhorn Hospital Research Grant, whose support is gratefully acknowledged. We also thank Dr. Danupon Nantajit for his kind assistance with the manuscript and Ms. Kamonwan Soonklang for her assistance with statistical

analysis.

Potential conflicts of interest

The authors declare no conflict of interest.

References

1. Levine RL, Gilliland DG. Myeloproliferative disorders. Blood 2008;112:2190-8.
2. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391-405.
3. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood 2000;96:3343-56.
4. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365: 1054-61.
5. Levine RL, Pardanani A, Tefferi A, Gilliland DG. Role of JAK2 in the pathogenesis and therapy of myeloproliferative disorders. Nat Rev Cancer 2007;7:673-83.
6. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemiavera. Nature 2005;434:1144-8.
7. Thiele J, Kvasnicka HM. The 2008 WHO diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis. Curr Hematol Malig Rep 2009;4:33-40.
8. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood 2005;106:2162-8.
9. Shirane S, Araki M, Morishita S, Edahiro Y, Takei H, Yoo Y, et al. JAK2, CALR, and MPL mutation spectrum in Japanese patients with myeloprolifera tive neoplasms. Haematologica 2015;100:e46-e48.
10. Ha JS, Kim YK, Jung SI, Jung HR, Chung IS. Correlations between Janus kinase 2 V617F allele burdens and clinicohematologic parameters in myeloproliferative neoplasms. Ann Lab Med 2012;32:385-91.
11. Chen S, Fei H, Zhang R, Xue Y, Pan J, Wu Y, et al. Analysis of JAK2(V617F) mutation in Chinese patients with myeloproliferative disorders. Am J Hematol 2007;82:458-9.
12. Shen YM, Chao HY, Zhang R, Feng YF, Cen JN, Yao L, et al.Quantitative assay for Janus kinase 2 (JAK2) mutation in Chinese patients withchronic myeloproliferative disorders. J Int Med Res 2009;37:37-46
13. Wang J, Zhang B, Chen B, Zhou RF, Zhang QG, Li J, et al. JAK2, MPL, and CALR mutations in Chinese Han patients with essential thrombocy themia. Hematology 2017;22:145-8.
14. Yeh YM, Chen YL, Cheng HY, Su WC, Chow NH, Chen TY, et al. High percentage of JAK2 exon 12 mutation in Asian patients with polycythemia vera. Am J Clin Pathol 2010;134:266-70.
15. Suksomyos N, Chanprasert S, Maunpasitporn C, Rojnuckarin P. Prevalence of JAK2V617F Mutation and Its Clinical Correlation in Thais with Myeloproliferative Neoplasm. Int J Biol Med Res 2012;3:1801-5.
16. Limsuwanachot N, Rerkamnuaychoke B, Chuncharunee S, Pauwilai T, Singdong R, Rujirachaivej P, et al. Clinical and hematological relevance of JAK2 V617F and CALR mutations in BCR-ABL-negative ET patients. Hematology 2017;22:599-606.
17. Kannim S, Thongnoppakhun W, Auewarakul CU. Two-round allele specific-polymerase chain reaction: a simple and highly sensitive method for JAK2V617F mutation detection. ClinChimActa 2009;401:148-51.
18. Lucia E, Martino B, Mammi C, Vigna E, Mazzone C, Gentile M, et al. The incidence of JAK2 V617F mutation in bcr/abl-negative chronic myelopro liferative disorders: assessment by two different detection methods. Leuk Lymphoma 2008;49:1907-15.
19. Frantz C, Sekora DM, Henley DC, Huang CK, Pan Q, Quigley NB, et al. Comparative evaluation of three JAK2V617F mutation detection methods. Am J Clin Pathol 2007;128:865-74.
20. Scott LM. The JAK2 exon 12 mutations: a comprehensive review. Am J Hematol 2011;86:668-76.
21. Park CH, Lee KO, Jang JH, Jung CW, Kim JW, Kim SH, et al. High frequency of JAK2 exon 12 mutations in Korean patients with polycythaemia vera: novel mutations and clinical significance. J Clin Pathol 2016;69:737-41.
22. Khuhapinant A, Lertchaisataporn K, Phikulsod P, Siritanaratkul N. Incidence and characteristics of polycythemia vera, essential thrombocythemia and primary myelofibrosis in Thailand: A 5-year

retrospective study [abstract]. 56th ASH Annual Meeting; 6th-9th December Moscone Center, San Francisco, USA. Blood 2014;124:5563.
23. Zhou J, Ye Y, Zeng S, Zhou Y, Mao Z, Song X, et al. Impact of JAK2 V617F mutation on hemogram variation in patients with non-reactive elevated platelet counts. PloS one. 2013;8(2):e57856.
24. Kittur J, Knudson RA, Lasho TL, Finke CM, Gangat N, Wolanskyj AP, et al. Clinical correlates of JAK2V617F allele burden in essential thrombocythemia. Cancer. 2007;109(11):2279-84.
25. Speletas M, Katodritou E, Daiou C, Mandala E, Papadakis E, Kioumi A, et al. Correlations of JAK2–V617F mutation with clinical and laboratory findings in patients with myeloproliferative disorders. Leuk Res. 2007;31(8):1053-62.
26. Wolanskyj AP, Lasho TL, Schwager SM, McClure RF, Wadleigh M, Lee SJ, et al. JAK2V617F mutation in essential thrombocythaemia: clinical associations and long term prognostic relevance. Br J Haematol. 2005;131(2):208-13.