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Determination of the *JAK2* V617F mutation in thrombosis patients

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ABSTRACT

Background: Janus kinase 2 (*JAK2*) gene mutation causes uncontrolled myeloproliferation independent of cytokines and abnormal formation of the endogenous erythroid colony. *JAK2* mutations were frequently observed in myeloproliferative neoplasms (MPNs), especially in polycythemia vera (PV) and essential thrombocythemia (ET). MPNs represent a risk factor for the development of thrombosis that is a significant cause of morbidity and mortality in patients.

Materials and methods: We aimed to study the correlation between *JAK2* mutation and thrombosis. Thirty-nine patients with a clinical diagnosis of thrombocytosis and Budd-Chiari syndrome were collected to determine the *JAK2* V617F mutation. Genomic DNA from all specimens was amplified and detected the presence of *JAK2* V617F mutation by AS-PCR.

Results: We demonstrated *JAK2* V617F mutation in both patients with a clinical diagnosis of thrombocytosis and Budd-Chiari syndrome. We found that 11 of 37 (29.7%) thrombocytosis patients had a *JAK2* V617F mutation. Moreover, one of two patients who represented as Budd-Chiari syndrome was positive for *JAK2* V617F mutation. In addition, *JAK2* V617F mutation was associated with thrombosis. However, further study in large series is needed to support this finding.

Conclusion: Determination of the *JAK2* V617F mutation may be helpful for screening latent or occult MPNs patients who have an occurrence of thrombosis to adjust the appropriate treatment for good patient outcomes.

Introduction

Human Janus kinase 2 (*JAK2*) is located on the short arm of chromosome 9 (9p24.1) and encodes JAK2 tyrosine kinase protein, which is critical for several cellular signaling pathways. During the past three decades, mutation on exon encoding Janus kinase 2 (*JAK2*) has been associated with the development of myeloproliferative neoplasms (MPNs). These include the *JAK2* V617F (exon 14) mutation, which is predominantly identified in the majority of polycythemia vera (PV) and essential thrombocythemia (ET).[1,2] Recently, our group demonstrated that *JAK2* V617F is positive in 95% of PV, 75% of ET, and 25% of primary myelofibrosis (PMF)[3]. Additionally, the mutation load (tumor burden) of *JAK2* V617F has been investigated and reported to be correlated with phenotypic differences among a subgroup of classical MPNs.[3-5] Moreover, several studies reported that mutation in *JAK2* genes is involved in leukemogenesis. These include translocation involving *JAK2* (e.g., *ETV6*::*JAK2* and *PAX5*::*JAK2*) in Philadelphia-

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liked acute leukemia.[6-8] These data highlighted the role of *JAK2* in homeostasis and the development of several blood cancers.

The common causes of mortality in several Philadelphia negative MPNs are recognized as arterial and venous thrombosis. These include portal vein thrombosis (PVT) and Budd-Chiari syndrome (BCS) (with hepatic vein thrombosis). Alteration of *JAK2* results in several physiological changes, including hypercellularity inducing blood viscosity, alteration of coagulation cascades and vessel wall, and disruption of cell-cell interaction/adhesion.[9] Recent reports demonstrated that polycythemia vera with *JAK2* mutation are associated with increased risks to thromboembolic events.[10-14] Additionally, there was evidence indicating that nearly half of BCS are diagnosed as chronic MPNs.[15-19] In this work, we aimed to investigate the prevalence of *JAK2* V617F mutation in patients with a clinical diagnosis of thrombocytosis and BCS.

Materials and methods

Patients and samples

Thirty-nine genomic DNA (gDNA) samples isolated from peripheral blood and bone marrow of patients who had a clinical diagnosis of thrombocytosis and Budd-Chiari syndrome were included in this study. gDNA was extracted by using QIAamp DNA Blood mini kit (Qiagen, Germany) and subsequently quantified by using Nanodrop 2000 spectrophotometer (Thermo Scientific, USA). All samples were stored at -20 °C refrigerator prior *JAK2* V617F genotyping. This work was approved by the ethic committee on human Rights related to research involving human subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand, and performed according to the principles of the Declaration of Helsinki (ID 03-58-51).

JAK2 V617F genotyping

Allele-specific polymerase chain reaction (AS-PCR) was performed according to the previously published protocol [1] to determine the *JAK2* V617F mutation statuses in all tested samples. Briefly, optimal PCR conditions were comprised of an initial denaturing at 95°C for 10 minutes, 30 cycles of 94 °C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. PCR was detained at 4° C until continuing to further steps. Specific nucleotide primer details are following; Forward outer (FO) 5'TCCTCAGAACGTTGATGGCAG3', Reverse outer (RO) 5' ATTGCTTTCCTTTTTCACAAGAT3', Forward wild type specific (Fwt) 5'GCATTTGGTTTTAAATTATGG AGTATATG3', Reverse mutant specific (Rmt) 5'GTTTTAC TTACTCTCGTCTCCACAAAA3', respectively. PCR products were subsequently analyzed and detected by using QIAxcel Advanced system (QIAGEN, Germany) system, which technology was based on capillary gel electrophoresis. The sample was automatically loaded into a gel matrix with dye-filled capillary. Then, the staining dye nucleic acid was detected by a photomultiplier detector and converted to a gel image and electropherogram by the QIAxcel ScreenGel Software. Positive samples later confirmed the *JAK2* V617F mutation status by using direct PCR sequencing (Sanger sequencing) as described by Baxter *et al*.[1]

Statistical analysis

The statistical analysis of obtained data was performed using the SPSS version 16.0 (SPSS Inc, Chicago, IL, U.S.A.) software. Chi-squared test was used for the comparison of categorical variables. A *p* value of less than 0.05 was considered to indicate statistical significance.

Results

Allele specific-PCR was performed to analyze *JAK2* V617F mutation in genomic DNA samples derived from 37 thrombosis and 2 Budd-Chiari syndrome patients. We found that *JAK2* V617F was positive in 11 of 37 (29.7%) patients with thrombocytosis. As expected, most ET with thrombosis (10 of 12, 83.3%) harbored *JAK2* V617F mutation. Interestingly, *JAK2* V617F mutation was positive in one out of two patients with Budd-Chiari syndrome (50%). To confirm *JAK2* V617F mutation, samples positive for *JAK2* V617F were subsequently analyzed by using the direct PCR sequencing technique (Figure 1). The baseline characteristics of patients and *JAK2* V617F mutation status are demonstrated in Table 1 and Table 2. While our data revealed that there was no statistic significant in *JAK2* V617F mutation status between patients with ET and BCS (p=0.325), BCS and other diseases (anemia, pure red cell aplasia (PRCA), middle cerebral artery (MCA) stroke, spinal stenosis, lung cancer, breast cancer, branch retinal vein occlusion (BRVO), portal vein thrombosis, cirrhosis, sinus venous thrombosis, and superior mesenteric vein (SMA) thrombosis) with thrombocytosis (p=0.526), *JAK2* V617F mutation was predominantly identified in ET compared with those who were characterized as other disorders with thrombocytosis (p<0.05) (Figure 2). Although limited in the sample size of BCS group, we observed that the platelet count in patients with thrombocytosis was not statistically significant to patients with BCS (p=0.0952) (Figure 3).

In summary, we demonstrated the beneficial use of *JAK2* V617F genotyping for the molecular classification of patients with thrombocytosis. *JAK2* V617F mutation was predominantly identified in patients with essential thrombocytosis. Moreover, half of the patient with BCS was positive for *JAK2* V617F. Our data further highlight the practical use of *JAK2* V617F mutation analysis for the classification of MPNs and differential diagnosis of MPNs with BCS from BCS with other diseases/complications.

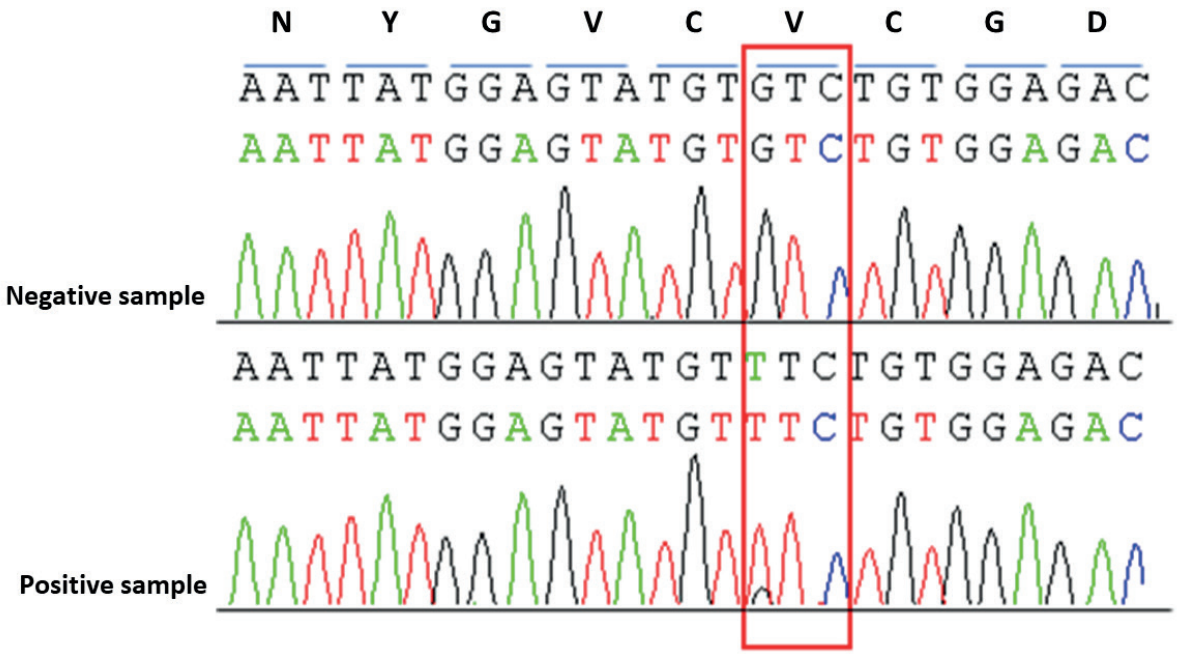

Figure 1. *Electropherogram of DNA sequencing data on JAK2 exon 14 from samples negative for JAK2 V617F (upper panel) and positive for JAK2 V617F (lower panel).*

Table 1 Baseline characteristics of the patients and *JAK2* V617F mutation status.

Diagnosis, Thrombocytosis	37/39 (94.8%)
ET with thrombocytosis	12/39 (30.7%)
Other disorders with thrombocytosis	25/39 (64.1%)
Budd-Chiari syndrome	2/39 (5%)
Sex (male/female)	11/28 (1:2.5)
Median age at thrombocytosis	57
Male	57
Female	53
JAK2 V617F positive	12/39 (30.7%)
ET with thrombocytosis	10/12 (83.3%)
Other disorders with thrombocytosis	1/25 (4.0%)
Budd-Chiari syndrome	1/2 (50.0%)

Table 2 *JAK2* V617F mutation status and platelet count in 37 thrombocytosis and 2 Budd-Chiari syndrome.

Patient	Diagnosis	*JAK2* V617F status	PLT x10^3/uL
HGL001	Thrombocytosis	Negative	ND
HGL002	Thrombocytosis	Negative	932
HGL003	ET with Thrombocytosis	Positive	360
HGL004	Thrombocytosis	Negative	569
HGL005	ET with Thrombocytosis	Positive	727
HGL006	Thrombocytosis	Positive	624
HGL007	ET with Thrombocytosis	Positive	519
HGL008	Thrombocytosis	Negative	483
HGL009	Thrombocytosis	Negative	1027
HGL010	ET with Thrombocytosis	Positive	1598
HGL011	Thrombocytosis	Negative	236
HGL012	ET with Thrombocytosis	Positive	745
HGL013	ET with Thrombocytosis	Positive	636
HGL014	ET with Thrombocytosis	Positive	2647
HGL015	ET with Thrombocytosis	Positive	492
HGL016	ET with Thrombocytosis	Positive	739
HGL017	Thrombocytosis	Negative	1103
HGL018	Thrombocytosis	Negative	586
HGL019	Thrombocytosis	Negative	ND
HGL020	Thrombocytosis	Negative	282
HGL021	Thrombocytosis	Negative	491
HGL022	Thrombocytosis	Negative	795
HGL023	Thrombocytosis	Negative	862
HGL024	Thrombocytosis	Negative	1046
HGL025	Thrombocytosis	Negative	1158
HGL026	Thrombocytosis	Negative	628
HGL027	Thrombocytosis	Negative	ND
HGL028	ET with Thrombocytosis	Negative	794
HGL029	Thrombocytosis	Negative	258
HGL030	Thrombocytosis	Negative	1240
HGL031	Thrombocytosis	Negative	1377
HGL032	Thrombocytosis	Negative	50
HGL033	Thrombocytosis	Negative	209
HGL034	Thrombocytosis	Negative	300
HGL035	Thrombocytosis	Negative	223
HGL036	ET with Thrombocytosis	Negative	900
HGL037	ET with Thrombocytosis	Positive	1079
HGL038	BCS	Negative	382
HGL039	BCS	Positive	80

Note: ET: essential thrombocythemia, BCS: Budd-Chiari syndrome, PLT: platelet, ND: no data.

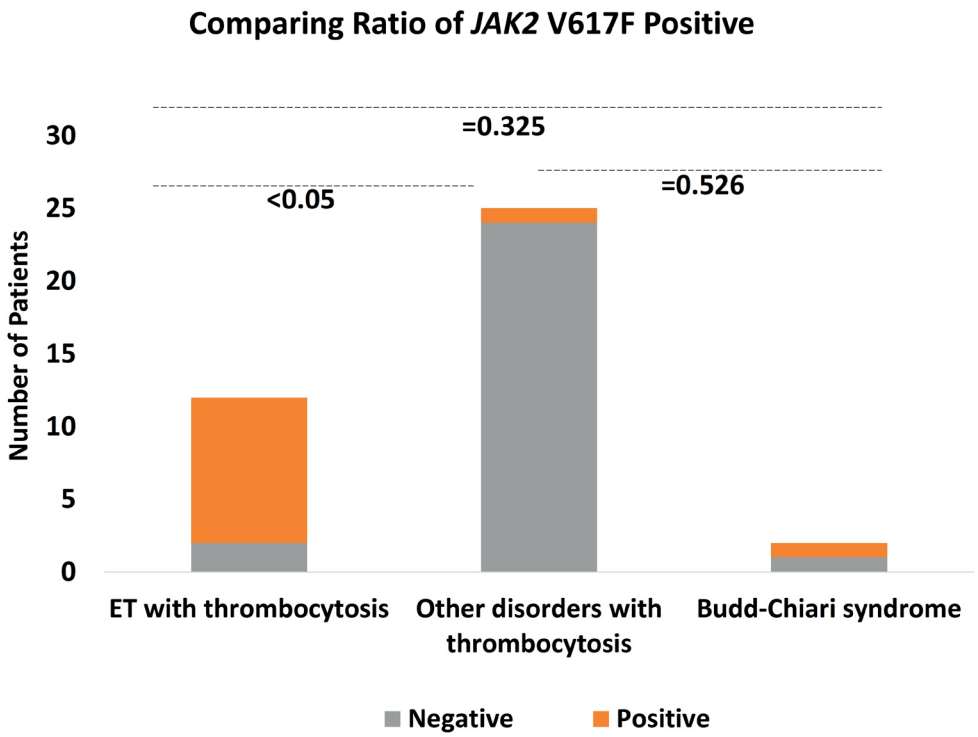

Figure 2. *Comparison of JAK2 V617F mutation status in a different group of patients with thrombocytosis.*

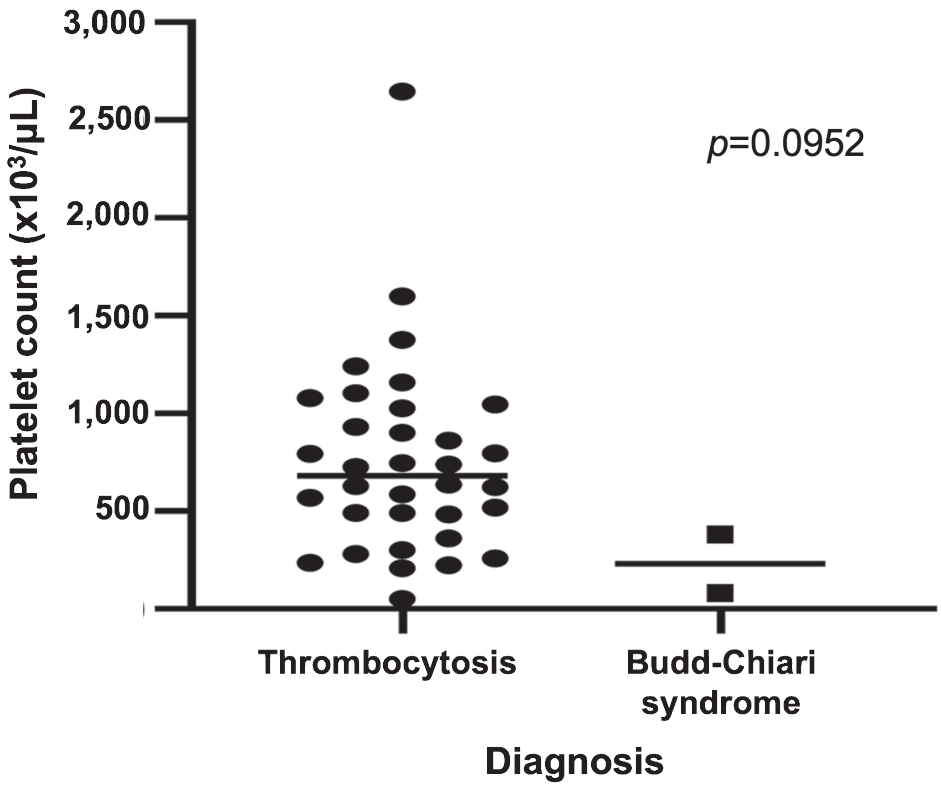

Figure 3. *Comparison of platelet number in patients with thrombocytosis and BCS.*

Discussion

Allele-specific PCR (AS-PCR) was applied as described previously for qualitative analysis of *JAK2* V617F mutation.[1] This assay has been widely used to detect *JAK2* V617F, which is predominantly positive in patients with myeloproliferative neoplasms (MPNs). Additionally, this assay has been expandable and used for the risk assessment of Budd-Chiari syndrome overt myeloproliferative disorders.[20,21] In this work, we used automatic capillary gel electrophoresis, QIAxcel (Qiagen), to replace traditional labor-intensive gel electrophoresis. This machine could perform genotyping analysis with highly sensitive sizing accuracy and is friendly used when compared with conventional slab-gel electrophoresis (Figure 4).

This data further confirmed the beneficial use of *JAK2* V617F as a genetic biomarker for the differential diagnosis of ET from other disorders with thrombocytosis.[22-24] Additionally, evidence indicates that the blood count of patients with MPNs is closely normal at BCS present.[25] Thus, the determination of *JAK2* V617F and other biomarkers for MPNs, such as calreticulin (*CALR*) and *MPL* are critical for the differential diagnosis of primary BCS and MPNs with BCS.

At present, there is less clarity about the incident of BCS in Thailand and worldwide. While the frequency of BCS among ethnic groups is consistent, the disease's observed frequency in Asian countries varies, which is a markedly high prevalence in Kathmandu, Nepal[26]. Perspective on the distribution of *JAK2* V617F in unselected patients with BCS, we compared our data to other groups working on the impact of *JAK2* V617F in BCS (Table 3). Similar to Yonal, Pinarbasi *et al*.[17] and Amarapurkar, Punamiya *et al*. 2008.[27], half of BCS patients were positive for *JAK2* V617F. In contrast, lower incidences of *JAK2* V617F mutation were reported in some studies which were conducted in a larger number of BCS samples.[21,28-30] In European countries, several studies indicated that *JAK2* V617F mutation was predominantly identified in BCS with underlying MPNs (nearly half of all BCS and about 90% of those with MPNs).[16,31-33] For a clearer view of the incidence of BCS in Thailand and the beneficial use of *JAK2* V617F for

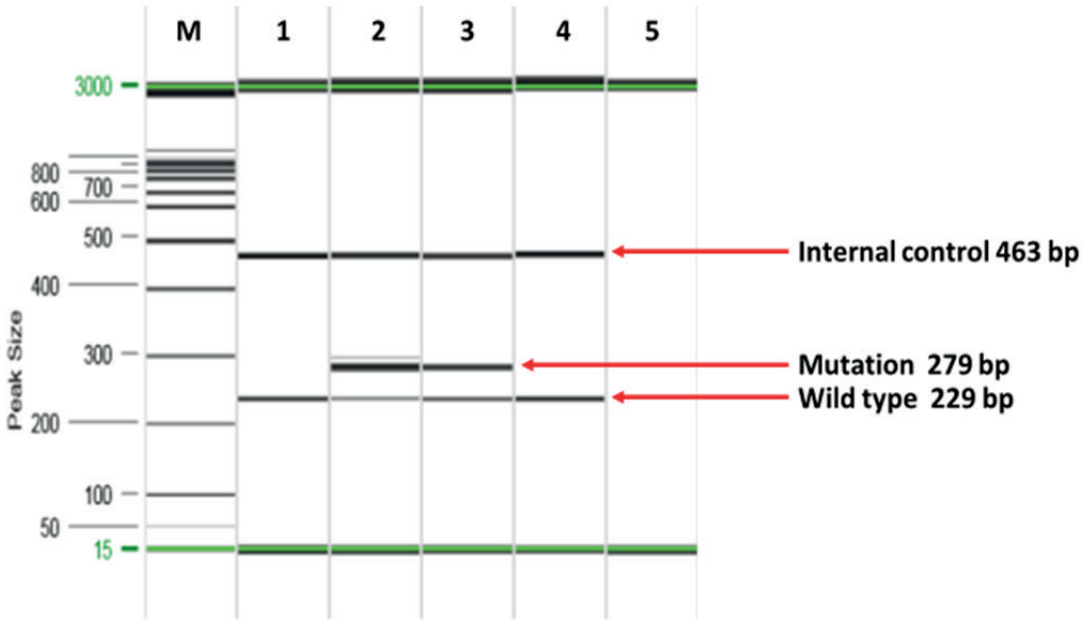

Figure 4. *Genotyping analysis of JAK2 V617F using automatic capillary gel electrophoresis. Lane M: size marker, lane 1: sample with a negative for JAK2 V617F, lane 2: sample with a positive for JAK2 V617F, lane 3: positive control, lane 4: negative control, lane 5: no template control, respectively.*

Table 3 Distribution of *JAK2* V617F in patients with unselected Budd-Chiari syndrome among different ethnic groups.

Study	Observed frequency (number of BCS samples)	Country
This study	50% (2)	Thailand
Qi[30]	5.2% (77)	China
Shetty[28,29]	8.8% (137)	India
Sakr[29]	19.1% (94)	Egypt
Karakose[21]	20.5% (31)	Turkey
Yonale[17]	50.0% (26)	Turkey
Amarapurkar[27]	47.8% (23)	India

categorizing the disease, more tested samples should be considered for improvement.

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