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Original Article

JAK2 V617F Mutation in Adult Taiwanese Patients with Essential Thrombocythemia: More Prevalent in Old Patients and Correlated with Higher Hemoglobin Level and Higher Leukocyte Count

Huan-Chau Lin [1,2], Caleb Gon-Shen Chen [1,2,3], Ming-Chih Chang [1], Wei-Ting Wang [2], Chen Wei Kao [2], An-Chi Lo [2], Nai-Wen Su [1,2], Yu-Cheng Chang [1], Yi-Hao Chiang [1], Kuei-Fang Chou [1], Po-Nien Liao [1], Guan-Jhe Cai [1], Hung-I Cheng [4], Johnson Lin [1], Yi-Fang Chang [1,2,5], Ruey-Kuen Hsieh [1], Ken-Hong Lim [1,2,5,6]*

[1] *Division of Hematology and Oncology, Department of Internal Medicine, Mackay Memorial Hospital, Taipei,* [2] *Laboratory of Good Clinical Research Center, Department of Medical Research, Mackay Memorial Hospital, Tamsui District, New Taipei City,* [3] *Institute of Molecular Medicine, National Tsing-Hua University, Hsin-Chu,* [4] *Division of Hematology, Department of Internal Medicine, Mackay Memorial Hospital, Hsin-Chu,* [5] *Mackay Medical College, New Taipei City,* [6] *Graduate Institute of Oncology, National Taiwan University College of Medicine, Taipei, Taiwan*

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SUMMARY

Background: Essential thrombocythemia (ET) is classified as a chronic myeloproliferative neoplasm. *JAK2* V617F mutation is found in about 50–60% patients with ET. We aim to determine the prevalence of *JAK2* V617F mutation and its association with phenotype in adult Taiwanese patients with ET.
Methods: In this combined retrospective and prospective study, adult ET patients, at least 18 years of age, were enrolled between November 2007 and September 2011. Genomic DNA was extracted from unsorted bone marrow and/or peripheral blood samples for the detection of *JAK2* V617F mutation by allele-specific polymerase chain reaction. The clinical and laboratory characteristics of all patients at the time of diagnosis or referral were determined retrospectively by chart review.
Results: A total of 82 patients were enrolled, and *JAK2* V617F mutation was detected in 55 patients (67.1%). *JAK2* V617F mutation was significantly more prevalent in old patients (36.4% vs. 14.8%, $p = 0.044$), and associated with higher hemoglobin level (median 13.7 vs. 12.8 g/dL $p = 0.012$) and higher white blood cell count at diagnosis (12.1×10^3 vs. $8.8 \times 10^3/\mu L$ $p = 0.015$). ET patients with the mutation also tend to have lower platelet count (median 902×10^3 vs. $1078 \times 10^3/\mu L$ $p = 0.051$). In a binary logistic regression model, only higher hemoglobin concentration was significantly associated with *JAK2* V617F mutational status (odds ratio 1.2 95% confidence interval 1.0–1.5; $p = 0.047$).
Conclusion: JAK2 V617F mutation in Taiwanese adult patients with ET has a high prevalence of 67.1% and is associated with old age, higher hemoglobin level, and higher leukocyte count.

1. Introduction

Essential thrombocythemia (ET) is classified as one of the *BCR-ABL1*-negative classic chronic myeloproliferative neoplasms (MPNs), which also include polycythemia vera and primary myelofibrosis[1]. ET is a clonal stem cell disorder and is characterized by excessive platelet production, usually without obvious panmyelosis feature, in the bone marrow[2–4]. Clinically, ET patients have a near-normal life expectancy[5,6]. Although a large number of ET patients may be asymptomatic at presentation, they may report vasomotor symptoms such as headache, lightheadedness, or erythromelalgia. Furthermore, some patients may experience thrombotic or hemorrhagic complications, which are related to qualitative and quantitative platelet alterations[7,8]. In some rare cases, disease transformation to polycythemia vera, secondary myelofibrosis, or acute leukemia may also be seen. Therefore, patients with ET require long-term clinical follow-up.

The diagnosis of ET is more challenging than other MPNs because no specific clinical, pathological, or molecular features are unique to ET, which is currently diagnosed by exclusion[2]. In the

* Correspondence to: Dr Ken-Hong Lim, Division of Hematology and Oncology, Department of Internal Medicine, Mackay Memorial Hospital, No. 92, Section 2, Chungshan North Road, Taipei 10449, Taiwan.
E-mail address: limkenhong@gmail.com (K.-H. Lim).

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2008 World Health Organization classification[2], diagnosis of ET requires the fulfillment of all the following four major criteria: (1) the clinical finding of a sustained and otherwise unexplained thrombocytosis ($\geq 450 \times 10^3/\mu L$); (2) the exclusion of other myeloid disorders including polycythemia vera, primary myelofibrosis, chronic myeloid leukemia, and myelodysplastic syndrome; (3) the proliferation of megakaryocytes without obvious atypia, and there is no granulocytic proliferation in the bone marrow; and (4) a clonal marker such as *JAK2* V617F or *TET2* mutation[9] or the exclusion of secondary thrombocytosis. Since its first discovery in 2005, the *JAK2* V617F mutation has important diagnostic and therapeutic implications in classic MPNs including ET[10–13]. *JAK2* V617F mutation results from a single-point mutation (G → T transversion) encoding a valine-to-phenylalanine substitution at position 617 in exon 14 of the *JAK2* gene. The autoinhibitory action of JH2 domain in *JAK2* gene is triggered in V617F mutation, which results in a constitutively activated *JAK2* tyrosine kinase. The prevalence of *JAK2* V617F mutation is reported in approximately 50–60% of patients with ET and primary myelofibrosis, and the incidence rate could rise up to more than 90% in polycythemia vera[7,14,15]. In the 2008 World Health Organization classification, the *JAK2* V617F mutation has been incorporated as one of the clonal marker, although not specific, for ET[2]. In addition, several *JAK2* inhibitors have been developed to target the JAK-STAT signaling pathway. The success of JAK inhibitor in the treatment of myelofibrosis has further proved that JAK-STAT signaling is important in the pathogenesis of MPNs[16,17].

The presence of *JAK2* V617F mutation in ET patients has been shown to be associated with some clinical and laboratory features, including a higher incidence of thrombosis, higher total white blood cell (WBC) counts and hemoglobin concentration, and lower platelet counts[16–21]. However, not all the above-mentioned features were found in each individual study. In the current study, we aim to determine the prevalence of *JAK2* V617F mutation by allele-specific polymerase chain reaction (AS PCR) in a cohort of Taiwanese adult patients with ET. The association between *JAK2* V617F mutation and clinical and laboratory characteristics at diagnosis or referral was also studied.

2. Patients and methods

2.1. Patients

This study was approved by the Institutional Review Board of the Mackay Memorial Hospital. Clinical samples were obtained between November 2007 and September 2011, and all patients provided written informed consent. The diagnosis of ET was based on the 2008 World Health Organization classification. Adult patients with ET who were at least 18 years of age and have received clinical follow-up at our hospital were enrolled retrospectively or prospectively. The clinical and laboratory characteristics of all patients at the time of diagnosis or referral were determined retrospectively by chart review.

2.2. Genomic DNA extraction

Unsorted bone marrow and/or peripheral blood WBCs were collected by using red blood cell lysis buffer. Peripheral blood mononuclear cells and/or granulocytes were isolated by usingFicoll-Paque™ PLUS (GE Healthcare, Salt Lake, UT, USA) density gradient separation according to the standard procedures. Genomic DNA was extracted using EasyPure Genomic DNA Spin Kit(Bioman, Taipei, Taiwan) for blood cells according to the manufacturer's instructions.

2.3. JAK2 V617F allele-specific polymerase chain reaction

The *JAK2* V617F AS primers that were used are as follows: specific forward primer (for the V617F allele mutant): 5′-AGCATTTGGTTTTAAATTATGGAGTATATT-3′, with an introduced mismatch at the third nucleotide from 3′-end (203-bp product); *JAK2* exon 14 control forward primer: 5′-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3′ (364-bp product); and the reverse primer: 5′-CTGAATAGTCCTACAGTGTTTTCAGTTTCA-3′[11]. The 12.5 μL *JAK2* AS PCR reaction volume incorporated 50 ng of template DNA, 6.25 μL of 2 × GoTaq Green Master Mix PCR buffer (Promega, Madison, CA, USA), and 1 μL from each of the 5 μM forward and reverse primers. The PCR amplification conditions were as follows: initial denaturation at 95°C for 5 minutes; 36 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 seconds; and final extension at 72°C for 10 minutes. The size and specificity of the products were confirmed by gel electrophoresis. The sensitivity of *JAK2* V617F AS PCR was determined to be 5% by serial dilution of *JAK2* V617F DNA (HEL, human erythroleukemia cell) in *JAK2*V617 wild-type control DNA (K562, human erythromyeloblastoid leukemia cell).

2.4. Statistical analyses

The association between *JAK2* V617F mutational status and clinical characteristics was calculated by the chi-square test or Fisher's exact test. The comparison between categorical and continuous variables was performed by the Mann–Whitney *U* test. Correlation between two continuous variables was determined with the nonparametric Spearman rank–order correlation coefficient. The binary logistic regression was used for multivariate analysis. Statistical significance was defined as a two-sided p value <0.05. Statistical analyses of the data were carried out by using the SPSS Statistics software (IBM, Armonk, NY, USA).

3. Results

The present study cohort enrolled 82 Taiwanese adult patients with ET, including 36 males and 46 females. The median age at diagnosis was 53.5 years (range 22–89 years). The median time from the diagnosis of ET to patient enrollment into the study was 2.9 years (range 0–22.0 years). The clinical and laboratory characteristics of these patients at diagnosis or referral are presented in Table 1. Overall, the *JAK2* V617F mutation was identified in 55 patients (67.1%). There were no significant differences in age at diagnosis, gender, disease duration, asymptomatic at presentation, the presence of vasomotor symptoms, or history of thrombotic and hemorrhagic complications between those with and those without the mutation. There were also no differences in gender, disease duration, asymptomatic at presentation, the presence of vasomotor symptoms, or history of thrombotic and hemorrhagic complications including both major and minor events between old and young patients. However, *JAK2* V617F mutation was significantly more prevalent in old patients (36.4% vs. 14.8%, $p = 0.044$). In addition, hemoglobin concentration and WBC count at diagnosis were significantly higher in ET patients with the mutation (median 13.7 vs. 12.8 g/dL, $p = 0.012$; 12.1×10^3 vs. $8.8 \times 10^3/\mu L$, $p = 0.015$, respectively). There was a trend that ET patients with the mutation had lower platelet count (median 902×10^3 vs. $1078 \times 10^3/\mu L$, $p = 0.051$).

The present study also analyzed the association between old age and the three laboratory parameters. Old age did not correlate with hemoglobin concentration, and WBC and platelet counts. Hemoglobin concentration also did not correlate with WBC count ($p = 0.6$), but it showed statistically significant negative correlation

Table 1
Clinical and laboratory characteristics at diagnosis or referral of 82 adult Taiwanese patients with essential thrombocythemia.

Characteristic	No. of patients ($n=82$) No. (%)	Age ≥65 y			*JAK2* V617F mutation		
		Yes ($n=24$) No. (%)	No ($n=58$) No. (%)	p	Positive ($n=55$) No. (%)	Negative ($n=27$) No. (%)	p
Time from diagnosis to enrolment, y ($n=75$) Median (Min–Max)	2.9 (0–22.0)	2.8 (0–8.0)	3.0 (0–22.0)	0.5[a]	3.0 (0–22.0)	2.6 (0–11.6)	0.9[a]
Age at diagnosis, y Median (Min–Max)	53.5 (22–89)	—	—	—	54 (22–89)	52 (25–79)	0.3[a]
Follow-up, mo Median (Min–Max)	44.8 (0.3–274.8)	41.4 (0.9–151.4)	46.0 (0.3–274.8)	0.3[a]	42.1 (0.3–274.8)	56.0 (2.1–158.3)	0.1[a]
Age ≥65 y	24 (29.3)	—	—	—	20 (36.4)	4 (14.8)	0.044
Male sex	36 (43.9)	13 (54.2)	23 (39.7)	0.2	25 (45.4)	11 (40.7)	0.7
Asymptomatic	34 (41.5)	10 (41.7)	24 (41.4)	1.0	20 (36.4)	14 (51.9)	0.2
Vasomotor symptoms	23 (28.0)	7 (29.2)	16 (27.6)	1.0[b]	18 (32.7)	5 (18.5)	0.2[b]
Dizziness	17 (20.7)	6 (25.0)	11 (19.0)	0.6[b]	13 (23.6)	4 (14.8)	0.4[b]
Headache	7 (8.5)	1 (4.2)	6 (10.3)	0.7[b]	5 (9.1)	2 (7.4)	1.0[b]
Acral paresthesia	3 (3.7)	2 (8.3)	1 (1.7)	0.2[b]	2 (3.6)	1 (3.7)	1.0[b]
History of thrombosis	13 (15.9)	5 (20.8)	8 (13.8)	0.5[b]	9 (16.4)	4 (14.8)	1.0[b]
Major arterial thrombosis	9 (11.0)	4 (16.7)	5 (8.6)	0.4[b]	6 (10.9)	3 (11.1)	1.0[b]
Major venous thrombosis	3 (3.7)	1 (4.2)	2 (3.4)	1.0[b]	2 (3.6)	1 (3.7)	1.0[b]
Minor thrombosis	2 (2.4)	0	2 (3.4)	1.0[b]	1 (1.8)	1 (3.7)	1.0[b]
History of hemorrhage	9 (11.0)	4 (16.7)	5 (8.6)	0.4[b]	6 (10.9)	3 (11.1)	1.0[b]
Major hemorrhage	7 (8.5)	4 (16.7)	3 (5.2)	0.2[b]	4 (7.3)	3 (11.1)	0.7[b]
Minor hemorrhage	2 (2.4)	0	2 (3.4)	1.0[b]	2 (3.6)	0	1.0[b]
Splenomegaly[c]	21 (25.6)	4 (16.7)	17 (29.3)	0.2	15 (27.3)	6 (22.2)	0.8[b]
Hemoglobin (g/dL) Median (Min–Max)	13.3 (4.5–17.9)	13.1 (9.6–17.6)	13.4 (4.5–17.9)	1.0[a]	13.7 (4.5–17.9)	12.8 (8.5–15.2)	0.012[a]
WBC ($\times 10^3/\mu L$) Median (Min–Max)	10.3 (4.8–29.9)	12.3 (6.0–29.9)	10.0 (4.8–29.3)	0.3[a]	12.1 (4.8–29.9)	8.8 (4.9–27.9)	0.015[a]
Platelet ($\times 10^3/\mu L$) Median (Min–Max)	944 (335–2834)	952 (339–1496)	944 (335–2834)	0.8[a]	902 (335–1931)	1078 (532–2834)	0.051[a]

Max = maximal; Min = minimal; WBC = white blood cell; — = not evaluated.
[a] Mann–Whitney *U* test; otherwise chi-square test was applied.
[b] Fisher's exact test.
[c] Including physical and imaging evaluation.

with platelet count (Spearman rho coefficient −0.237, $p=0.032$). In a binary logistic regression model, old age, hemoglobin concentration, and WBC count were selected as covariates and *JAK2* V617F mutational status as a dependent variable. Only higher hemoglobin concentration remained significantly associated with *JAK2* V617F mutational status in this model (odds ratio 1.2, 95% confidence interval 1.0–1.5; $p=0.048$).

The median follow-up time for all 82 patients was 44.8 months from diagnosis (range 0.3–274.8 months). During this period, two ET patients with *JAK2* V617F mutation underwent disease transformation to acute myeloid leukemia and expired at 147.08 and 103.9 months after diagnosis, respectively. All the remaining 80 patients were still alive without transformation. No significant difference was seen in the degree of disease transformation into acute myeloid leukemia between the two mutational groups in this cohort. Due to very few survival events, median survival was not reached and could not be compared between different groups.

4. Discussion

In the present study, the prevalence of *JAK2* V617F mutation in Taiwanese adult patients with ET was determined as 67%. Recently, the prevalence of *JAK2* V617F mutation in Chinese or Taiwanese patients with ET was also reported by several studies[21–25], and the incidence rate is ranged from 34% to 66%. When compared to other studies, the incidence of *JAK2* V617F mutation in Taiwanese was comparable with that of other ethnic groups, although our result was among the highest ones (Table 2)[14,16–18,21,24–36]. The difference in the prevalence of *JAK2* V617F mutation may result from the difference in sample size, the use of different source of samples for testing, and the different methods used for detecting mutation. Since *JAK2* V617F mutation has an important diagnostic implication in MPNs including ET, we recommend the use of a more sensitive method such as AS PCR for its detection.

The presence of *JAK2* V617F mutation has been found to be associated with several clinical and hematologic abnormalities in ET patients in this study. In a univariate analysis, old age (≥65 years old), higher hemoglobin, and higher leukocyte count were significantly associated with *JAK2* V617F mutation. The degree of the association between lower platelet counts and *JAK2* V617F mutation in the present study was only at borderline. Similar findings were also reported in several studies (Table 2). Regarding the association of old age, Randi et al[28] have found that the percentage of mutated patients and the *JAK2* V617F allele burden increased progressively with age. These phenotypic effects of *JAK2* V617F mutation are very distinctive features in patients with ET. Based on the mutational status of *JAK2* V617F, ET patients can be separated into two distinct subtypes. The *JAK2* V617F mutated patients who are generally older have higher hemoglobin level and WBC count, and lower platelet count. In a multivariate analysis using binary logistic regression model, only higher hemoglobin level remained significantly associated with *JAK2* V617F mutation. The presence of *JAK2* V617F mutation may skew the presenting phenotype and the bone marrow histology of ET toward a more "erythremic" feature[32]. This was consistent with the finding that the prevalence of *JAK2* V617F mutation was highest (more than 90%) in patients with polycythemia vera. It is likely that the acquisition of *JAK2* V617F mutation may have a distinct biological effect on ET patients, although this is a late genetic event in some ET patients[37].

The association between *JAK2* V617F mutation and thrombosis is a controversial issue; in our study, such an association was not found. However, in a recent reviewed including 21 studies involving patients with ET, Lussana et al[38] found that the risk of both venous and arterial thrombosis was significantly increased in

Table 2
Studies evaluating phenotypic relevance of *JAK2* V617F allele according to its presence/absence in patients with ET[a,26].

Reference no.	This study	27	24	25	21	28	14	29	16	18	17	30	31	32	33	34	35	36
Authors	Lin et al	Cho et al	Wong et al	Hsiao et al	Wong et al	Randi et al	Campbell et al	Antonioli et al	Wolanskyj et al	Cheung et al	Kittur et al	Pemmaraju et al	Finazzi et al	Rudzki et al	Heller et al	Alvarez-Larran et al	Vannucchi et al	Antonioli et al
Year	2012	2009	2011	2007	2008	2011	2005	2005	2005	2006	2007	2007	2007	2007	2006	2007	2007	2008
Patient no.	82	108	102	53	95	132	776	130	150	60	176	80	179	59	50	126	639	260
V617F-positive patient no. (% of total)	55 (67)	61 (56.5)	35 (34)	35 (66)	60 (63)	87 (65.9)	414 (53)	74 (57)	73 (49)	29 (48)	96 (55)	38 (47)	103 (57)	34 (57)	24 (48)	44 (43)	382 (60)	165 (63)
Hematologic characteristics																		
Higher Hb and/or Htc	Yes	No	No	Yes	No	—	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Higher WBC	Yes	Yes	Yes	Yes	No	—	Yes	No	Yes	No	Yes	No	Yes	Yes	No	No	Yes	Yes
Lower Plt count	No	No	No	No	Yes	—	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Clinical characteristics																		
Older age	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	No	Yes	Yes	—	—	Yes	—	Yes	Yes
Gender	No	No	Yes	No	No	No	No	No	No	No	No	—	No	—	No	No	No	No
Disease duration	No	Yes	No	—	No	—	No	No	No	No	—	Yes	No	—	No	—	No	No
Pruritus	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	No	No
Systemic symptoms	No	—	No	—	—	—	—	—	No	—	—	—	—	—	—	—	No	No
Palpable splenomegaly	—	—	—	—	—	—	Yes	—	No	—	No	—	—	—	—	—	Yes	No
Major CV events	No	No	—	Yes	Yes	No	No	No	No	Yes	No	No	Yes	—	Yes	No	No	No
Microvessel disease	—	—	—	—	—	—	—	—	No	—	No	No	—	—	No	—	—	Yes
Major hemorrhages	No	No	—	No	No	—	No	No	No	—	—	—	—	—	—	No	No	No
CHT treatment	—	No	No	—	No	—	—	—	No	—	—	—	No	—	No	—	No	No
Evolution to MF	—	No	—	—	—	—	No	—	No	—		—	No	—	—	No	No	—
Evolution to AML	—	No	—	—	—	—	No	—	No	—		—	No	—	—	—	No	—
Evolution to PV	—	—	—	—	—	—	Yes	—	Yes	—	No	—	—	—	—	Yes	No	—
Overall survival	—	No	—	—	—	—	No	—	Yes	—	No	—	—	—	—	—	—	—

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Higher Hb/Htc, WBC or platelet count refers to the comparison of patients harboring the *JAK2* V617F mutation versus wild-type patients. The line indicates that no information is available.
AML = acute myelogenous leukemia; CHT treatment = percentage of patients who received chemotherapy; CV = cardiovascular events; ET = essential thrombocythemia; Hb = hemoglobin; Htc = hematocrit; MF = post-ET myelofibrosis; Plt = platelet count; WBC = white blood cell count.
[a] Only studies reporting at least 50 total patients were included.

JAK2 V617F mutated patients. Nevertheless, the presence of *JAK2* V617F mutation has not yet been listed as a risk factor for thrombosis in ET[39]. Currently, ET patients are considered at high risk if they are older than 60 years of age or if there is a history of previous thrombosis.

There were several limitations in the present study, including the use of different sources of blood samples for the detection of *JAK2* V617F mutation (some of the samples were collected while the patients were being treated with cytoreductive therapy such as hydroxyurea) and a relatively short follow-up period in this cohort. Nevertheless, we have found a high prevalence of *JAK2* V617F mutation and its association with characteristic phenotypes including old age, higher hemoglobin level, and higher leukocyte count in Taiwanese adult patients with ET.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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