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Correlative study between the JAK2V617F mutation and thrombosis in patients with myeloproliferative neoplasm

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ABSTRACT. In this study, we investigated the correlation between the JAK2V617F mutation and thrombosis in patients with myeloproliferative neoplasm (MPN) using real-time fluorescence quantitative PCR. The incidence of thrombus was monitored and blood and coagulation were routinely assayed in patients with MPN. The JAK2V617F mutation was found in 8/68 individuals in the control group (11.8%); it was expressed in 44/68 patients with MPN (64.7%), suggesting that the rate of this mutation was significantly higher in patients with MPN than that in the control group. Twenty-six MPN patients (38.2%) showed symptoms of thrombosis; MPN patients with thrombosis showed a significantly higher rate of the JAK2V617F mutation, were of a greater age, and had higher blood pressure than MPN patients without thrombosis. In

addition, the white blood cells (WBC) (21.98 ± 1.95) and platelets (364.68 ± 97.72) were significantly higher in patients, expressing the mutated gene, with polycythemia vera than in the patients without the mutation. The WBC (32.89 ± 4.25) and hemoglobin (161.92 ± 16.19) were significantly increased in the essential thrombocythemia patients with gene mutation compared with the patients without mutation. MPN patients showed higher blood clotting ability than the control subjects; moreover, MPN patients with the JAK2V617F mutation showed higher blood clotting ability than those without the mutation. The findings of this study indicate that the JAK2V617F mutation is correlated with the incidence of thrombosis, and analysis of this mutation has important clinical significance in the diagnosis and treatment of MPN.

Key word: Myeloproliferative neoplasm; JAK2V617F mutation; Thrombosis

INTRODUCTION

Myeloproliferative neoplasma (MPN) is a clonal disorder of hematopoiesis, characterized by the production of mature-like cells within the blood stream. Classic BCR/ABL-negative chronic myeloproliferative disorders (MPDs) encompass three clonal diseases that arise in pluripotent hematopoietic stem cells and share clinical, hematological, and biological features: polycythemia vera (PV) (Gómez et al., 2016; McMullin et al., 2016), essential thrombocythemia (ET), and primary myelofibrosis (PMF) (Tefferi, 2010). The clinical features of MPN patients include excessive proliferation of red blood cells and platelets with splenomegaly, bleeding, bone marrow fibrosis, leukemic transformation tendency, and thrombosis. Thromboembolism seriously affects the quality of life of patients with MPN, and is the key cause of mortality (Ziakas, 2008). Early diagnosis of thrombosis is essential to reduce the mortality of patients with MPN. In 2008, the World Health Organization (WHO) proposed that the JAK2V617F mutation could be one of the diagnostic criteria of classic BCR/ABL PMN (Tefferi and Vardiman, 2008). In this study, we assayed the JAK2V617F mutation rate and the incidence of thrombosis in MPN patients, and explored the correlation between the JAK2V617F mutation and the incidence of thrombosis.

MATERIAL AND METHODS

Patients

Patients with MPN admitted to Hematology and Oncology Department of our hospital were selected between February 2014 and February 2015. A total of 68 patients with MPN, including 31 male and 37 female patients, were recruited to this study (age range, 41-73 years; average age, 59). The patients were classified into three types of MPD based on the diagnostic classification criterion proposed by the WHO in 2008 (Tefferi and Vardiman, 2008), as follows: 37 patients with ET (17 males and 20 females; average age, 49); 23 patients with PV (12 males and 11 females; average age, 57); and 8 with PMF (2 males and 6 females; average age, 65).

The control group was composed of 68 individuals, among whom 21 were patients with other blood diseases such as leukemia, anemia, or infection mononucleosis, and 47 were healthy individuals (30 males and 38 females; age range: 38-73 years; average age, 57).

Written informed consent was obtained from all participants prior to enrollment in the study according to the principles of the Declaration of Helsinki; the study design was approved by the Shandong University Ethics Committee.

JAK2V617F mutation test

Peripheral blood was collected from patients with MPN and the control individuals (2-3 mL each) in tubes containing EDTA anticoagulant, and stored in a -80°C refrigerator. Genomic DNA was extracted and amplified by PCR, according to the protocols detailed by the manufacturer in a commercial JAK2V617F mutation detection kit (Koyee Bio-Technology Co., Ltd., Shanghai, China), using a LightCycler 480 fluorescent PCR gene amplification detector (Roche, Indianapolis, IN, USA). The amplified DNA products were separated by agarose gel electrophoresis, and examined by an ultraviolet reflection transmission analyzer. Samples positive for the mutated JAK2V617F gene were sequenced by Adicon (Beijing, China).

Detection of thrombosis in patients with MPN

Factors influencing thrombosis, including the age, gender, obesity status, and blood pressure, in MPN patients were recorded and analyzed. Patients were selected according to the following criteria: age ≥60 years; systolic pressure ≥139 mmHg and/or diastolic pressure ≥89 mmHg without using an antihypertensive drug; or blood pressure ≤140/90 mmHg using an antihypertensive drug. Body mass index is the standard of obesity. Thrombotic disease was recorded for 1 year after MPN diagnosis.

Routine blood and coagulation testing

Peripheral blood (3-5 mL each) was collected in EDTA anticoagulant for routine blood testing using 10 mL sodium citrate, an AutoVue Innova kit (Johnson & Johnson, USA), and an Automatic Blood Coagulation Analysis Instrument SF-8000 (Succeeder, China).

Statistical analysis

Statistical evaluation was performed on the SPSS 21.0 software platform (SPSS, Inc., Chicago, IL, USA). All data are reported as means ± standard deviation (SD) of multiple iterations. The Student paired *t*-test was used to identify differences between groups; categorical variables were compared using the chi-squared test. A P value <0.05 was considered statistically significant; multivariate logistic regression analysis was performed.

RESULTS

Screening for the JAK2V617F mutation

The JAK2V617F mutation was detected in both patients with MPN and the control

subjects. The results revealed the presence of the mutation in 44 of 68 patients with MPN (64.7%; 24 patients with ET, 17 with PV, and 3 patients with PMF). However, the JAK2V617F mutation was found only in 8 of 68 subjects in the control group (11.8%), suggesting that the rate of JAK2V617F mutation in patients with MPN was significantly higher than that in the control group. The incidences of the JAK2V617F genetic mutation are summarized in Table 1.

Table 1. Incidence of JAK2V617F genetic mutation.

Group	Number	JAK2V617F		Incidence of mutation
		Positive	Negative	
MPN	68	44	24	64.7%*
ET	37	24	13	64.9%
PV	23	17	6	73.9%
PMF	8	3	5	37.5%
Control	68	8	60	11.8%

*P < 0.05 *vs* the control group. MPN: myeloproliferative neoplasm; ET: essential thrombocythemia; PV: polycythemia vera; PMF: primary myelofibrosis.

Incidence of thrombosis in patients with MPN

Twenty-six of 68 patients (38.2%) with MPN displayed symptoms of thrombosis (including 7 MPN patients with thrombotic disease), among which 13, 12, and 1 were patients with ET, PV, and PMF, respectively. The type of thrombosis in these MPN patients ranged from arterial thrombosis (N = 15; 9 with cerebral thrombosis, 3 with coronary thrombosis, and 3 patients with superior mesenteric artery thrombosis) and phlebothrombosis (N = 9; 5 patients with lower extremity deep vein thrombosis, 3 with portal vein thrombosis, and 1 with splenic vein thrombosis), to arteriovenous thrombosis (N = 2; cerebral thrombus + portal vein thrombosis and cerebral thrombosis + lower extremity deep vein thrombosis).

Relationship between thrombosis and JAK2V617F in patients with MPN

The rate of JAK2V617F mutation, age, and blood pressure status of MPN patients with thrombosis was significantly higher than that of MPN patients without thrombosis (P < 0.05). However, no significant difference was found in the age and obesity status between patients with and without thrombosis. Thrombus rates in patients with MPN are shown in Table 2.

Table 2. Thrombus rates in patients with myeloproliferative neoplasm.

Group	Age (≥60 years)	Gender		Obesity	Blood pressure	JAK2V617F
		Male	Female			
Thrombus	21*	14		11	23*	
Non-thrombus	17	24		25	9	

*P < 0.05 *vs* the non-thrombus group.

Routine blood and coagulation testing in patients with MPN

Compared with the PV patients without gene mutation, white blood cells (WBC) and platelets were significantly increased in the patients with mutation. Compared with the ET

patients without gene mutation, WBC and hemoglobin were significantly increased in the patients with mutation (Table 3). Moreover, the MPN patients showed higher blood clotting ability than the control group, and the JAK2V617F mutative patients showed higher blood clotting ability than the patients without mutation among the MPN patients (Table 4).

Table 3. Blood results from patients with myeloproliferative neoplasm.

Group	WBC (10^9/L)	PLT (10^9/L)	HGB (g/L)
JAK2V617F positive			
ET	32.89 ± 4.25*	881.41 ± 320.23	161.92 ± 16.19*
PV	21.98 ± 1.95*	364.68 ± 97.72*	178.33 ± 17.21
PMF	14.36 ± 3.01	179.85 ± 33.56	142.12 ± 4.19
JAK2V617F negative			
ET	16.11 ± 2.48	693.52 ± 237.29	144.13 ± 20.62
PV	13.98 ± 1.34	216.39 ± 89.74	137.96 ± 13.21
PMF	13.25 ± 1.17	156.74 ± 18.32	128.93 ± 1.37

*P < 0.05 *vs* the JAKV617F negative group. ET: essential thrombocythemia; PV: polycythemia vera; PMF: primary myelofibrosis; WBC: white blood cell; PLT: platelet; HGB: hemoglobin.

Table 4. Results of the routine coagulation test of patients with myeloproliferative neoplasm (MPN).

Group	PT	APTT	TT	FIB (g/L)
MPN patient	16.64 ± 3.98*	58.91 ± 21.19*	21.01 ± 3.53*	2.81 ± 1.22
JAK2V617F positive	16.13 ± 3.41#	62.29 ± 23.55#	20.43 ± 4.12#	2.19 ± 1.52
JAK2V617F negative	14.97 ± 2.02	49.83 ± 8.36	18.36 ± 2.13	2.18 ± 0.71
Control	13.98 ± 0.89	33.69 ± 5.71	17.09 ± 2.32	2.41 ± 0.82

*P < 0.05 *vs* the control group; #P < 0.05 *vs* JAK2V617F negative group. PT: prothrombin time; APTT: activated partial thromboplastin time; TT: thrombin time; FIB: fibrinogen.

DISCUSSION

MPN is a clonal hematopoietic stem cell disease characterized by sustained proliferation of bone marrow cells (Tefferi et al., 2007). In this disease, blood cells proliferate significantly because of the excessive proliferation of, and apoptosis inhibition in, bone marrow hematopoietic stem cells. Blood cell proliferation causes an increase in hematocrit, blood viscosity, and platelet count, leading to serious complications such as bleeding and thrombus embolism. Carobbio et al. (2007) reported that leukocytes promote thrombosis. Neutrophils and platelets form complexes to regulate the expression of platelets, such as P-selectin and tissue factor, thereby promoting the activation of vascular endothelial cells. A previous study suggested that mortality caused by thrombosis accounted for 35-70% of the total mortality in MPN patients (Park et al., 2013). Therefore, it is a condition that generates significant complications for MPN patients. Our results showed that 26/68 (38.2%) MPN patients, including 7 with thrombotic disease, showed thrombosis.

Finazzi et al. (2007) reported that the incidence of thrombus in patients with the JAK2V617F mutation is higher than that in patients without this mutation. Kralovics et al. (2005) also reported that patients expressing the JAK2V617F mutation are more susceptible to complications such as thrombosis. Therefore, the JAK2V617F mutation is the most remarkable genetic mutation in MPN. The JAK2V617F mutation was first identified in patients with MPN in 2005 (Reikvam and Tiu, 2012; Bogani et al., 2013), and

was proposed as a diagnostic criterion for BCR/ABL-negative MPN by the WHO in 2008. However, the pathogenesis of the JAK2V617F mutation in classic BCR/ABL-negative MPN remains unclear. The current opinion leans towards the JAK2V617F mutation theory. The high frequency point mutations in JAK2 exon 12 (G mutates into T at site 1849) are believed to lead to a valine changing into a phenylalanine at site 617 of the JAK2 kinase domain structure. This change causes instability in, and abnormal activation of, the JH2 structure and further activation of transcription in the cell proliferation gene via activation of the JAK/STAT signal transduction pathways, resulting in sustained cellular proliferation (Harrison et al., 2012; Evrot et al., 2013; Hsiao et al., 2013). In this study, we showed that the mutation was found in 44 of 68 patients with MPN (64.7%), including 24 ET, 17 PV, and 3 PMF patients. These results indicate the high incidence rate of the JAK2V617F mutation in patients with MPN.

In this study, we evaluated several factors affecting the incidence of thrombosis in patients with MPN, such as the age, gender, obesity status, blood pressure, and the JAK2V617F mutation. Our investigation revealed that patients with thrombosis showed significantly higher rates of the JAK2V617F mutation, were of a greater age, and had higher blood pressure than patients without thrombosis had. These results suggest that the JAK2V617F mutation is an important risk factor for the incidence of thrombosis in MPN patients. The results of the routine blood test showed a significant increase in WBCs in patients with PV and ET expressing the mutation, indicating that WBCs may play an important role in the incidence of thrombosis in MPN patients. The results of the routine coagulation test revealed that MPN patients with the JAK2V617F showed a significantly higher blood clotting ability than those without the mutation. This result suggests that patients with MPN show abnormal coagulant function, which should be investigated in future studies.

There are a few limitations to this study. First, the study population was relatively small, which may have limited the statistical power for the identification of differences between groups. Second, although we have demonstrated the correlation between the JAK2V617F mutation and the incidence of thrombosis, we did not clarify the underlying mechanism, which is worthy of further study. Third, the results of this observational study should be confirmed by larger clinical trials in multiple clinical centers.

In conclusion, JAK2V617F is an important diagnostic standard in patients with MPN; therefore, the detection of this mutation has important clinical significance. JAK2V617F participates in the development of MPN, and is correlated with thrombotic disease in MPN patients. Therefore, testing for the JAK2V617F mutation could help identify the incidence of thrombotic disease in MPN patients, and could be helpful in the clinical diagnosis and treatment of MPN.

Conflicts of interest

The authors declare no conflict of interest.

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