Original Article

JAK2 V617F, MPL W515L and JAK2 Exon 12 Mutations in Chinese Patients with Primary Myelofibrosis

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ABSTRACT

Objective: JAK2 V617F, MPL W515L and JAK2 exon 12 mutations are novel acquired mutations that induce constitutive cytokine-independent activation of the JAK-STAT pathway in myeloproliferative disorders (MPD). The discovery of these mutations provides novel mechanism for activation of signal transduction in hematopoietic malignancies. This research was to investigate their prevalence in Chinese patients with primary myelofibrosis (PMF).

Methods: We introduced allele-specific PCR (AS-PCR) combined with sequence analysis to simultaneously screen JAK2 V617F, MPL W515L and JAK2 exon 12 mutations in 30 patients with PMF.

Results: Fifteen PMF patients (50.0%) carried JAK2 V617F mutation, and only two JAK2 V617F-negative patients (6.7%) harbored MPL W515L mutation. None had JAK2 exon 12 mutations. Furthermore, these three mutations were not detected in 50 healthy controls.

Conclusion: MPL W515L and JAK2 V617F mutations existed in PMF patients but JAK2 exon 12 mutations not. JAK2 V617F and MPL W515L and mutations might contribute to the primary molecular pathogenesis in patients with PMF.

Key words: Primary myelofibrosis; JAK2 V617F; MPL W515L; JAK2 exon 12; mutation

INTRODUCTION

Primary myelofibrosis (PMF) is a clonal stem cell disorder characterized by chronic myeloproliferation, atypical megakaryocytic hyperplasia, and bone marrow fibrosis. The disorder manifests clinically as anemia, splenomegaly due to extramedullary hematopoiesis (EMH), leukoerythroblastosis, and constitutional symptoms. Based on the seminal editorial by Dameshek in 1951[1], PMF is classified as one of the prototypic myeloproliferative disorders (MPD), along with polycythemia vera (PV) and essential thrombocytosis (ET). In 2005, using different approaches, several independent groups virtually simultaneously reported on a recurrent point mutation in the JAK2 tyrosine kinase (JAK2 V617F) in several MPD, including PV, ET, and PMF[2-6]. The allelic frequency of JAK2 V617F is upwards of 90% in PV, and roughly 50% in ET and PMF, as estimated by sensitive detection methodologies[7, 8]. The mutation has been described as a specific marker of MPD, neither detectable in healthy subjects nor in reactive proliferations[9, 10], although it could be present in rare cases of other clonal myeloid disorders[11, 12]. While JAK2 V617F is the predominant disease-associated allele in MPD, approximately 10% of patients meeting the clinical criteria for PV and 50% for ET and PMF do not present such mutations, and genetic analysis is still under progress to elucidate the responsible oncogenic events. More recently, two other related mutations have been described in patients with JAK2 V617F-negative PMF/ET (MPL W515L/K mutation) or PV (JAK2 exon 12 mutations).

The mutation involved in MPL 515, either W515L or W515K (MPL W515L/K), occurs in 8.5% of JAK2 V617F-negative ET patients[13], about 10% of JAK2 V617F-negative PMF patients[14-16], and 0% of PV[15]. Scott, et al. recently described mutations of JAK2 exon 12 in JAK2 V617F-negative patients with PV or

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idiopathic erythrocytosis[17]. Their findings have been confirmed by other studies[18-20].

Though JAK2617F and MPLW515L/K mutations in Chinese patients has been reported in several studies, JAK2 exon 12 mutations has been rarely researched in Chinese PMF patients. To investigate the prevalence of JAK2 V617F, MPL W515L and JAK2 exon 12 mutations in Chinese patients with PMF, we introduced allele-specific PCR (AS-PCR) and gene sequencing to screen these mutations in 30 PMF patients.

MATERIALS AND METHODS

Patients

Between January 2006 and June 2008, a total of 30 Chinese PMF patients and 50 healthy controls were enrolled in the present study. Nineteen patients were male and 11 were female (male to female ratio, 1.7), and the median age at diagnosis was 54 years (range, 32–77 years). All samples were taken on the same date for analysis of JAK2 V617F MPL W515L and JAK2 exon 12 mutations. A total of 2 ml peripheral blood samples of PMF patients were obtained at the time of initial diagnosis with informed consent, and genomic DNA was isolated with the use of the QIAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The diagnosis of PMF was established according to 2001 World Health Organization (WHO) diagnostic criteria[21]. The patients were confirmed as BCR/ABL fusion transcript negative in contrast to CML as BCR/ABL positive.

JAK2 V617F and MPL W515L Mutations Analysis

AS-PCR was used for screening JAK2 V617F point mutation and MPL W515L mutation. PCR amplification was performed according as we previously described[22, 23]. For JAK2 V617F mutation, the electrophoresis showed that mutant allele is two bands including 203 bp and 364 bp but wild-type only 364 bp. For MPL W515L mutation, the electrophoresis showed that mutant allele is two bands including 279 bp and 409 bp but wild-type only 409 bp. The positive samples were all amplified only with the outer primer pair again and confirmed by sequence analysis.

JAK2 Exon 12 Mutation Analysis

We performed AS-PCR using DNA from total peripheral blood. The primers included JAK2 exon 12 control primers, and primers specific for the alleles containing the K539L mutation (leading to the replacement of lysine at position 539 with a leucine), the N542-E543del mutation (causing the deletion of asparagine at position 542 and glutamic acid at position 543), the F537-K539delinsL mutation (leading to the replacement of phenylalanine at position 537 through lysine at position 539 by a single leucine), or the H538QK539L mutation (causing a substitution of glutamine for histidine at position 538 and leucine for lysine at position 539) (Table 1)[17]. Each 25 μl PCR reaction solution contained approximately 25 ng of DNA template, 12.5 μl 2× taq PCR Master Mix (TIANGEN, KT201), and 0.4 μl (10 mmol/L) common reverse primer as well as 0.4 μl (10 mmol/L) two forward primers. PCR cycling parameters were: 94˚C for 4 min; 35 cycles of 94˚C for 30 s, 62˚C for 30 s and 72˚C for 60 s; followed by 72˚C for 10 min. The electrophoresis, on an ethidium bromide- impregnated 1.5% agarose gel, showed that mutant allele has two bands including 348 bp/342 bp and 496 bp but wild-type only 496 bp. The positive samples were all amplified only with the outer primer pair again and confirmed by sequence analysis using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Bio-Systems, Foster City, California, USA) on an ABI Prism 3700 DNA analyzer (Perkin-Elmer BioSystems). In addition, sequencing was also performed in patients whose AS-PCR electrophoresis had only one band in 496 bp.

Statistical Analysis

All statistical analyses were performed using the SPSS program for Windows (version 15.0) (SPSS Inc., Chicago, IL, USA). Comparisons between continuous variables were performed using the *t*-test, while categorical variables using the Chi-square test. The continuous variables included age and the counts of platelets and megakaryocytes in bone marrow. The categorical variables included gender and the incidence of thrombosis. $P<0.05$ was considered statistically significant.

RESULTS

Through AS-PCR combined with sequence analysis, samples from 30 patients with PMF were screened for the presence of JAK2 V617F, MPL W515L and JAK2 exon 12 mutations. Fifteen PMF patients (50.0%) carried JAK2 V617F mutation (Figure 1), and only two JAK2 V617F-negative patients (6.7%) harbored MPL W515L mutation (Figure 2). None had JAK2 exon 12 mutations. Furthermore, these three mutations were not detected in 50 healthy controls. There were no significant differences in median age at presentation or gender between JAK2 V617F-positive and JAK2 V617F-negative PMF patients ($P>0.05$). Seven JAK2 V617F-positive patients had thrombosis, but only three JAK2 V617F-negative PMF patients had