Prevalence of MPL W515L/K Mutations in Taiwanese Patients With Philadelphia-negative Chronic Myeloproliferative Neoplasms

Chien-Hui Lieu1,4, Yu-Jie Shen1, Wei-Chih Lai1, Wen-Hui Tsai6, Hui-Chi Hsu2,3,5*

1Department of Biotechnology and Laboratory Science in Medicine, School of Biomedical Science and Engineering, and 2Institute of Physiology, National Yang-Ming University School of Medicine; 3Division of General Medicine and Division of Hematology and Oncology, Department of Medicine, Taipei Veterans General Hospital; Departments of 4Education and Research, and 5Medicine, Taipei City Hospital Yang-Ming Branch, and 6Department of Respiratory Therapy, Taipei Medical University, Taipei, Taiwan, R.O.C.

Background: The discovery of Janus kinase 2 (JAK2)-V617F has provided important insight into the pathogenesis of Philadelphia-negative chronic myeloproliferative neoplasms (Ph-negative MPNs); however, the etiology of JAK2-V617F-negative Ph-negative MPN remains unidentified. MPL W515L and MPL W515K (MPL W515L/K) are 2 gain-of-function mutations, which have been found in some Ph-negative MPN patients from Western countries. However, little is known about the incidence of these mutations in Taiwanese Ph-negative MPN patients.

Methods: We determined the MPL sequence of DNA samples from 105 patients, including 88 patients with Ph-negative MPNs and 17 with myelodysplastic syndrome, using polymerase chain reaction amplification of the cytokine receptor MPL exon 10 sequence.

Results: All the patients were normal at codon 515 regardless of their JAK2 status.

Conclusion: The MPL W515L/K mutations are rare in Taiwanese patients with Ph-negative MPNs. [J Chin Med Assoc 2010;73(10):530–532]

Key Words: essential thrombocytopenia, idiopathic myelofibrosis, MPL mutation, myeloproliferative disorders, polycythemia vera

Introduction

The Philadelphia-negative chronic myeloproliferative neoplasms (Ph-negative MPNs) are classified by the World Health Organization into polycythemia vera (PV), essential thrombocytopenia (ET), idiopathic myelofibrosis (IMF), and chronic myeloid leukemia together with rarer subtypes such as chronic neutrophilic leukemia, hypereosinophilic syndrome and chronic eosinophilic leukemia.1 These disorders share many features, such as hypercellularity of the marrow, unstimulated overproduction of 1 or more lineages of blood cells, increased risk of thrombosis and bleeding, and they may spontaneously convert to acute leukemia, and also to marrow fibrosis.2 These clonal hematopoietic malignancies are considered to arise from transformation of pluripotent stem cells, and this leads to an increased production of erythrocytes, leukocytes and/or platelets.3,4 The pathogenesis of Ph-negative MPNs has been investigated but has remained unclear. Several studies have demonstrated a breakthrough finding by detection of an activating mutation V617F in Janus kinase 2 (JAK2) in some Ph-negative MPN patients. This shed new light on the pathogenesis of Ph-negative MPNs.5–8 This JAK2 V617F mutation was found in 70–90% of patients with PV, in 35–70% with ET, and in 30–50% with IMF. Molecular and clinical evidence has shown that the JAK2 V617F mutation has a direct causal role in the pathogenesis of Ph-negative MPNs.9,10
The discovery of JAK2V617F has provided important insight into the pathogenesis of PV, ET and IMF, but the etiology of JAK2V617F-negative Ph-negative MPN remains unknown. Recently, sequence analysis of the erythropoietin receptor, thrombopoietin receptor and granulocyte-colony stimulating factor receptor in JAK2-negative PV, ET and IMF led to the discovery of a somatic tryptophan-to-leucine substitution mutation at the transmembrane juxtamembrane junction of the thrombopoietin receptor MPL (MPLW515L).11 In addition, a small number of patients have an alternate mutation at codon 515, which results in a tryptophan-to-lysine substitution (MPLW515K).12 Expression of MPLW515L in recipient mice results in a myeloproliferative disease with similarities to human IMF, including reticulin fibrosis, megakaryocytic hyperplasia, splenomegaly, and extramedullary hematopoiesis. Similar to JAK2V617F, MPLW515L is an acquired mutation that induces cytokine-independent growth and thrombopoietin hypersensitivity, and results in constitutive phosphorylation of JAK2, STAT3, STAT5, AKT, and ERK. The frequency of the gain-of-function mutation MPLW515K/L was reported to be 1% in ET and 5% in IMF in the USA.12 Some patients may harbor coexisting JAK2V617F and MPLW515K/L mutations.13,14 Although MPLW515L/K mutations are much less common than JAK2V617F, the detection of MPLW515L in MPN patients may provide valuable information. We have determined the frequency of the JAK2V617F mutation in Taiwanese patients,15 but the frequency of MPLW515L mutations has not yet been determined. Therefore, we conducted this study to determine the prevalence of MPLW515K/L mutations in Taiwanese patients with Ph-negative MPN and myelodysplastic syndrome.

Methods

Genomic polymerase chain reaction (PCR)
This study was approved by the institutional ethics committee of Taipei Veterans General Hospital, Taiwan. Peripheral blood samples were collected from a total of 110 patients and informed consent was obtained from all patients.

Total DNA was isolated from leukocytes from patients’ peripheral blood using a QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s instructions. The human MPL exon 10 sequence was amplified from 0.5–1.0 μg of genomic DNA by PCR using specific primers (forward primer: 5’ TGGGCCGAAGTCTGACCCTTT 3’; reverse primer: 5’ ACAGACCGACCAAGAACCCCTCTG 3’).10 The reaction was performed for 30 cycles with a standard protocol and an annealing temperature of 60°C. The products were resolved on 2.5% agarose gels and visualized by ethidium bromide staining. The specific band was purified and sequenced by an ABI PRISM 3100 Genetic Analyzer (ABI Applied Biosystems, Foster City, CA, USA).

Results

We extracted genomic DNA from the peripheral blood of 105 patients. Of these patients, 88 were Ph-negative MPN (Table 1) and 17 had myelodysplastic syndrome. Diagnosis of ET, PV and IMF followed the criteria of the Polycythemia Vera Study Group.16,17

After PCR amplification of the cytokine receptor MPL exon 10 and subsequent sequencing, we found

Table 1. Characteristics of 88 patients with Philadelphia-negative chronic myeloproliferative neoplasms

<table>
<thead>
<tr>
<th>Disease subtype</th>
<th>PV (n = 33)</th>
<th>ET (n = 49)</th>
<th>IMF (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>22/11</td>
<td>27/22</td>
<td>5/1</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>68 ± 13</td>
<td>67 ± 14</td>
<td>72 ± 16</td>
</tr>
<tr>
<td>Median disease duration (mo)</td>
<td>55 (14–273)</td>
<td>35 (10–180)</td>
<td>124 (70–264)</td>
</tr>
<tr>
<td>WBC (×10^9/L)</td>
<td>12.1 ± 6.1</td>
<td>8.9 ± 4.8</td>
<td>8.9 ± 7.1</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>14.2 ± 2.1</td>
<td>12.6 ± 2.2</td>
<td>9.0 ± 1.8</td>
</tr>
<tr>
<td>Platelets (×10^9/L)</td>
<td>442 ± 338</td>
<td>583 ± 227</td>
<td>56 ± 32</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>7.7 ± 3.3</td>
<td>15.5 ± 11.6</td>
<td>18.4 ± 12</td>
</tr>
<tr>
<td>BM fibrosis (+)</td>
<td>37.5%</td>
<td>40%</td>
<td>100%</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>55%</td>
<td>53%</td>
<td>100%</td>
</tr>
<tr>
<td>CAD or HT (+)</td>
<td>54.5%</td>
<td>42.9%</td>
<td>33.3%</td>
</tr>
<tr>
<td>JAK2 V617F (+)</td>
<td>28/33 (85%)</td>
<td>29/49 (59%)</td>
<td>2/6 (33%)</td>
</tr>
<tr>
<td>Thrombosis/hemorrhage</td>
<td>7/1</td>
<td>13/1</td>
<td>0/1</td>
</tr>
</tbody>
</table>

PV = polycythemia vera; ET = essential thrombocythemia; IMF = idiopathic myelofibrosis; WBC = white blood cell count; Hb = hemoglobin; BM = bone marrow; CAD = coronary artery disease; HT = hypertension; JAK2 = Janus kinase 2.
that all the patients showed a normal codon at allele 515.

Discussion

Our data showed that 88 patients with Ph-negative MPN did not have MPLW515L/K mutations. Consistent with our results, Hsiao et al also reported that MPLW515L/K mutations could not be found in 53 Taiwanese patients with ET.18 Pardanani et al suggested that MPLW515 mutations are disease-specific.12 In the USA, they are prevalent in 5–9% of patients with IMF and in 1% of ET patients.11,12 Similarly, Hu et al reported that 7% of American patients with IMF showed these 2 mutations.19 However, we could not detect MPLW515L/K mutations in 6 patients with IMF. It appears that the genetic origins of IMF represent the culmination of multiple genetic and possibly epigenetic events. A number of laboratories have suggested that additional genetic events might play a role in this process.20–22 Comparative genomic hybridization studies have shown that gains of cytogenetic material occur in more than 50% of patients with IMF and most commonly involve gains of 9p, 2q, 3p, chromosome 4, 12q and 13q.22 Furthermore, Dingli et al have identified an unbalanced translocation between chromosomes 1 and 6 with specific breakpoints (1,6) that they believe are highly specific to IMF.20 These chromosomal sites may harbor additional genes that play a role in the origins of IMF. However, further studies with more patients are needed to determine the incidence and role of MPLW515L/K mutations in Taiwanese patients with IMF.

Acknowledgments

This study was supported by a grant from the Veterans General Hospital and University System of Taiwan (VGHU ST96-P7-38).

References