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Differences in Hematological and Clinical Features Between Essential Thrombocythemia Cases With *JAK2*- or *CALR*-Mutations

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Dear Editor,

Essential thrombocythemia (ET) is a myeloproliferative neoplasm (MPN) that primarily involves the megakaryocytic lineage, and is characterized by increased numbers of large, mature megakaryocytes in bone marrow as well as sustained thrombocytosis. Mutations in *JAK2* or *calreticulin* (*CALR*) are present in about 50% and 25% of patients with ET, respectively, and these mutations are thought to drive MPN [1]. *CALR* and *JAK2* mutations are mutually exclusive in MPNs [1]. Compared with patients with *JAK2*-mutated ET, patients with *CALR*-mutated ET have lower Hb levels and lower numbers of granulocytes, but higher numbers of platelets [2-6]. The *CALR*-mutated patients also have a lower incidence of thrombosis during their clinical course. Genetic background such as race may influence the risk of thrombosis, and recent study reported that Japanese ET patients with *JAK2* mutation had a higher cumulative incidence of thrombosis than those with *CALR* mutations, although the differences were not significant [6]. Therefore, we analyzed the impact of *JAK2* and *CALR* mutations on clinical features and thrombotic events in Japanese patients with ET.

One hundred forty-nine patients diagnosed as having ET at the Department of Gastroenterology and Hematology, University of Miyazaki or other participating institutions in Japan between February 2007 and December 2012, according to the 2008 or 2001 WHO diagnostic criteria [7, 8] were included in this study. The coding sequences of *JAK2* (exon 14), *MPL* (exon 10), and *CALR* (exons 9) in them were examined by Sanger sequencing. *JAK2* mutation was examined at diagnosis, and the status of *MPL* and *CALR* mutation was evaluated for this study by using frozen DNA samples. Their clinical and hematological features based on their genetic mutations were retrospectively analyzed. Univariate analyses comparing variables between ET patients with *JAK2* mutation and those with *CALR* mutation were done with the *t* test for continuous variables, and Pearson's χ^2 test with Yates' continuous correction for 2×2 tables. The number of leukocytes was compared with the Wilcoxon rank-sum test because data were heavily skewed. This study was approved by the Research Ethics Committee of the University of Miyazaki.

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The median age of the 149 patients with ET was 59 yr (range, 8-94), and 71 subjects (48%) were male. The *JAK2* V617F mutation was detected in 78 (52%) patients, *CALR* exon 9 mutations in 39 (26%), and *MPL* W515L/K mutations in 5 (3%). The remaining 27 cases (18%) exhibited none of these three mutations and were considered triple-negative MPNs (Fig. 1A). Our result confirmed previous findings regarding mutational status in patients with ET; about a half of the patients with ET harbored a *JAK2* V617F mutation, and about one-quarter of the patients with ET had a *CALR* mutation. The proportion of patients with triple-negative ET was 18% in our study, which was similar to the range of 10-19% reported in previous studies [2-4, 6].

Various *CALR* mutations were found in exon 9. Type 1 mutations, a 52-bp deletion in exon 9, were the most common and were observed in 23 cases (59%) (Fig. 1B). Type 2 mutations, a 5-bp insertion in exon 9, were identified in seven cases (18%), while nine other types of *CALR* exon 9 mutations occurred in one case each. All *CALR* mutations resulted in +1 bp frameshifts and led to a novel C-terminal peptide sequence. The newly formed C-terminus of *CALR* lacked the KDEL motif and contained positively charged amino acids (AAs) such as lysine and arginine instead of negatively charged AAs such as aspartic acid and glutamic acid.

We compared hematological and clinical features of patients with *JAK2* mutations and *CALR* mutations (Table 1). Patients with *CALR*-mutated ET displayed a unique phenotype; compared with patients with *JAK2*-mutated ET, they were younger and predominantly male. They also exhibited lower Hb levels, but higher platelet counts. These characteristics are identical to the previous results [2-6], and indicate that patients with *CALR*-mutated ET display a phenotype favoring megakaryopoiesis as opposed to the skewed erythropoiesis found in patients with *JAK2*-mutated ET. Mutant CALR was reported to augment STAT5 activation in the presence of MPL, but not in the presence of the EPO receptor [9]. Increased STAT5 activation by mutant CALR in cells that express MPL might cause the preferential expansion of the megakaryocyte lineage. In contrast to previous reports, there was no difference in neutrophil counts between patients with *JAK2*-mutated and *CALR*-mutated ET in this study, but patients with *CALR*-mutated ET had lower leukocyte alkaline phospha-

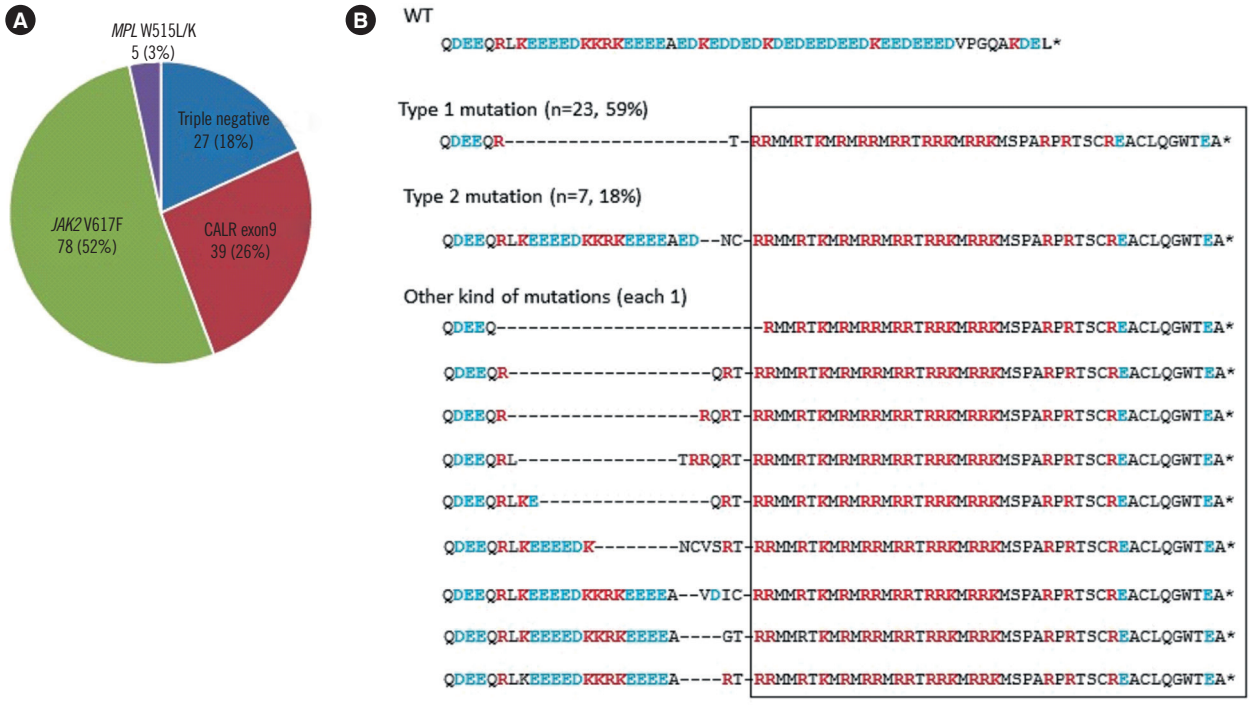

Fig. 1. Mutational status in 149 patients with ET. (A) Frequency of *JAK2, CALR*, and *MPL* mutations in ET. Eighteen percent of patients were negative for all three kinds of mutations. (B) Analysis of the *CALR* C-terminal amino acid (AA) sequence. In addition to the common Type 1 and Type 2 mutations, nine types of *CALR* exon 9 mutations were observed in one case each. All mutation types resulted in +1 bp frameshifts and led to a novel C-terminal peptide sequence, in which the KDEL motif was absent and positively charged AAs (red text) were substituted for negatively charged AAs (blue text).
Abbreviations: ET, essential thrombocythemia; WT, wild type.

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Table 1. Clinical and hematological features of patients with essential thrombocythemia with respect to *JAK2* and *CALR* mutation status

Variable	*JAK2* V617F mutation (n=78)	*CALR* mutation (n=39)	*P*
Age median (yr, range)	63.5 (21-85)	52 (8-94)	<0.05
Sex (M/F)	30/48	28/11	<0.01
WBC ($\times 10^9$/L, median, range)	10.6 (4.5-46.7)	9.0 (3.4-22.4)	n.s.
Granulocytes ($\times 10^9$/L, mean±SD)	8.2±5.5	6.9±4.5	n.s.
Hb (g/L, mean±SD)	143±21	136±16	<0.05
Plt ($\times 10^9$/L, mean±SD)	947±525	1143±599	<0.05
Ferritin (μg/L, mean±SD)	85.0±75.4	158.8±87.2	<0.01
Erythropoietin (IU/L, mean±SD)	11.8±7.0	36.4±9.9	<0.01
Leukocyte alkaline phosphatase score (mean±SD)	270.6±86.1	166.6±52.9	<0.01
Splenomegaly (presence/examined, %)	23/52 (45%)	11/24 (46%)	n.s.
Thrombosis during follow-up (presence/examined, %)	21/77 (26%)	3/39 (7.7%)	<0.05

Distribution of variables was evaluated by using the Kolmogorov-Smirnov test, and all except for the number of WBC were distributed normally.
Abbreviations: WBC, white blood cell; Plt, platelet; n.s., no significance.

tase scores compared with patients with *JAK2*-mutated ET.

In the course of ET, thrombotic events occurred in 26% of patients with *JAK2*-mutated ET versus 7.7% of patients with *CALR*-mutated ET. Consistent with previous reports [2-5], patients with *CALR*-mutated ET in this study had a lower risk of thrombosis than patients with *JAK2*-mutated ET. The widely accepted risk factors for thrombosis in ET were age >60 yr old and a history of thrombosis. In addition, elevated white blood cell count (>11×10^9/L) and presence of *JAK2* mutations were reported to be associated with thrombotic events, while elevated platelet count (>$1{,}000\times 10^9$/L) was associated with lower arterial thrombotic risk [10]. Clinical features observed in patients with *CALR*-mutated ET, including younger age, higher platelet count, and lack of *JAK2* mutation might contribute to their lower risk of thrombosis.

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Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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