JAK2 V617F, *MPL*, and *CALR* Mutations in Korean Patients with Essential Thrombocythemia and Primary Myelofibrosis

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Mutations in the calreticulin gene, *CALR*, have recently been discovered in subsets of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF). We investigated Korean patients with ET and PMF to determine the prevalence, and clinical and laboratory correlations of *CALR/JAK2/MPL* mutations. Among 84 ET patients, *CALR* mutations were detected in 23 (27.4%) and were associated with higher platelet counts ($P = 0.006$) and lower leukocyte counts ($P = 0.035$) than the *JAK2* V617F mutation. Among 50 PMF patients, *CALR* mutations were detected in 11 (22.0%) and were also associated with higher platelet counts ($P = 0.035$) and trended to a lower rate of cytogenetic abnormalities ($P = 0.059$) than the *JAK2* V617F mutation. By multivariate analysis, triple-negative status was associated with shorter overall survival (HR, 7.0; 95% CI, 1.6-31.1, $P = 0.01$) and leukemia-free survival (HR, 6.3; 95% CI, 1.8-22.0, $P = 0.004$) in patients with PMF. The type 1 mutation was the most common (61.1%) type among all patients with *CALR* mutations, and tended toward statistical predominance in PMF patients. All 3 mutations were mutually exclusive and were never detected in patients with other myeloid neoplasms showing thrombocytosis. *CALR* mutations characterize a distinct group of Korean ET and PMF patients. Triple-negative PMF patients in particular have an unfavorable prognosis, which supports the idea that triple-negative PMF is a molecularly high-risk disease.

Keywords: *CALR*; *JAK2* V617F; *MPL*; Thrombocythemia, Essential; Primary Myelofibrosis

INTRODUCTION

Although the *JAK2* V617F gain-of-function mutation has a pivotal role in the diagnosis of Philadelphia chromosome-negative myeloproliferative neoplasms (Ph⁻MPN), it only occurs in 50%-60% in patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF). Gain-of-function mutation in *MPL*, which is another molecular marker for ET and PMF, has been reported in 5%-10% of patients with these diseases with nonmutated *JAK2* (1). Therefore, the diagnosis of other patients with findings suggestive of ET or PMF but negative for *JAK2* and *MPL* mutations, must be based on the clinical exclusion of reactive thrombocytosis and histopathological examinations that focus on morphologic changes in megakaryocytes or bone marrow fibrosis. Histopathological evaluation is inherently susceptible to interobserver variation, requires an experienced hematopathologist, and lacks standardization. Therefore, it is very interesting that 60% to 88% of ET and PMF patients negative for *JAK2* V617F and *MPL* mutations have been found to harbor novel mutations of the calreticulin gene, *CALR* (1-8).

Calreticulin is a functionally complex Ca²⁺-binding protein that is localized primarily to the endoplasmic reticulum (9-11). All *CALR* mutations reported so far are located in exon 9 and are somatic insertions or deletions. There are two major variants: type 1 (L367fs*46), resulting from a 52-bp deletion, and type 2 (K385fs*47), from a 5-bp (TTGTC) insertion (1-8). In particular, the clinical course of PMF patients with *CALR* alterations has been found to be more indolent than the courses of patients with the *JAK2* V617F mutation and patients who are triple-negative (5, 6).

The aim of this study was to determine the prevalence, biological characteristics, and clinical correlations of these novel *CALR* mutations, in addition to the well-established *JAK2* V617F and *MPL* mutations, in a single-center cohort of patients with ET and PMF.

MATERIALS AND METHODS

Patients

The study included 150 patients seen at the Asan Medical Center, Seoul, Korea from January 2003 to April 2013. There were 84 patients with ET, 50 patients with PMF, 7 patients with post-ET or post-polycythemia vera (PV) myelofibrosis, and 9 patients with other myeloid neoplasms with thrombocytosis, as follows: 4 refractory anemia with ring sideroblasts associated with marked thrombocytois, 1 refractory anemia with multilineage dys-

plasia, 1 myelodysplastic syndrome with isolated del(5q), 1 chronic myeloid leukemia, 1 myelodysplastic/myeloproliferative neoplasm, unclassifiable, and 1 acute myeloid leukemia. It should be stressed that the study patients, who were selected, were primarily those with available archived samples. Furthermore, patients with available data for *JAK2* and *MPL* mutations were preferentially analyzed.

The median age of study patients was 60 yr (range, 19-90 yr); and 74 (49.3%) patients were male. ET, PMF, and other myeloid malignances were diagnosed based on 2008 World Health Organization (WHO) criteria. Post-ET or -PV myelofibrosis and leukemic transformation were also diagnosed according to 2008 WHO criteria. Major thromboembolic events at presentation or in the 2 preceding years or anytime during follow-up were recorded if definitely documented. Bone marrow aspirations and biopsies were independently reviewed by 2 hematopathologists who were blinded to the patient's mutation profile. Discrepant cases were resolved by consensus between the 2 hematopathologists. The clinical and laboratory parameters and cytogenetic findings at the time of initial presentation were reviewed. Karyotypes were designated as unfavorable based on the Dynamic International Prognostic Scoring System (DIPSS) risk categorization, as described previously; and included a complex karyotype (the presence of 3 or more distinct numeric or structural cytogenetic abnormalities) or 1 or 2 abnormalities that included +8, -7/7q-, i(17q), inv(3), -5/5q-, 12p- or 11q23 rearrangement (12).

Mutation and cytogenetic analysis

Study samples were either stored DNA extracted from peripheral blood or bone marrow of patients at initial presentation, or genomic DNA extracted from archived bone marrow smears according to a standard protocol and our laboratory's internal guidelines. The *JAK2* V617F mutation was assessed using a polymerase chain reaction (PCR)-based amplification refractory mutation system, as previously described (13). The *MPL* W515L/K mutations were assessed by real-time PCR (Real-Q *MPL* W515L/K Screening Kit, BioSewoom Inc., Seoul, Korea) according to the manufacturer's instructions. All positive samples by real-time PCR were subsequently analyzed by Sanger sequencing. Exon 10 of *MPL* was amplified using the following primers: F, 5´-TTCTGTACATGAGCATTTCATCA-3´ and R, 5´-GACAGGCTGTGT-GTGTGTACCTCT-3´. The *CALR* mutations were assessed by PCR followed by Sanger sequencing. Exon 9 of *CALR* was amplified using the following primers: F, 5´-GAGGAGTTTGGCAACGAGAC-3´and R, 5´-AACCAAAATCCACCCCAAAT-3´. The PCR conditions consisted of initial denaturation step at 94℃ for 5 min; followed by 35 cycles of 94℃ for 45 sec, 57℃ for 30 sec, and 72℃ for 1 min; and a final extension step at 72℃ for 10 min. Purified PCR fragments were sequenced. Cytogenetic analysis was performed using conventional G-banding techniques.

Statistical analysis

Differences in the distributions of continuous variables between categories were analyzed using either the Wilcoxon rank-sum test or the Kruskal-Wallis test. Patient groups with nominal values were compared using the chi-square test or Fisher exact test. Overall survival (OS) was calculated from the date of diagnosis of ET or PMF to date of death (uncensored) or last contact (censored). Date of leukemic transformation replaced date of death (uncensored) to evaluate leukemia-free survival (LFS). OS and LFS were plotted using Kaplan-Meier curves and compared by a log-rank test. The Cox proportional hazard regression model was used for multivariate analysis. P values < 0.05 were considered to indicate statistically significant differences. Statistical analyses were performed using MedCalc program version 12.4.0.0 (MedCalc Software, Acacialaan, Belgium).

Ethics statement

This study was approved by the institutional review board of the Asan Medical Center (2014-0974). The board waived informed consent from participants. The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Frequencies and distribution of mutations

CALR mutations were detected in 36 patients (25.5%) of patients with ET or PMF or post-ET or PV myelofibrosis. Among these patients, type 1 (L367fs*46), type 2 (K385fs*47) and other types of mutations were detected in 22 (61.1%), 10 (27.8%), and 4 (11.1%) patients, respectively. The type 1 mutation was found more frequently in patients with PMF than the type 2 mutation, with marginal statistical significance ($P = 0.076$, Table 1).

Of the 84 patients with ET, 43 (51.2%) harbored the *JAK2* V617F mutation, 23 (27.4%) had *CALR* mutations, and 1 (1.2%) had *MPL* W515 mutation. Seventeen (20.2%) patients were negative for all 3 mutations (Fig. 1A). ET patients with *CALR* mutations accounted for 57.5% of patients that had nonmutated *JAK2* and *MPL*. Of the ET patients with *CALR* mutations, 11 (47.8%) had type 1 mutations, 9 (39.1%) had type 2 mutations, and 3 (13.0%) had other types of mutations (Table 1).

Of the 50 patients with PMF, 27 (54.0%) harbored the *JAK2*

Table 1. Frequency of the *CALR* mutation subtypes detected in this study of Korean patients with essential thrombocythemia and primary myelofibrosis

Type	ET (n = 23)	PMF (n = 11)	Post-ET or PV MF (n = 2)
Type 1	11 (47.8%)	9 (81.8%)	2 (100.0%)*
Type 2	9 (39.1%)	1 (9.1%)	0
Others†	3 (13.0%)	1 (9.1%)	0

*These patients had post-ET myelofibrosis, not post-PV myelofibrosis; †Other types included c.1091_1124del (p.E364fs*55), c.1101_1105delAGG (p.K368del), c.1113_1143del (p.E372fs*48), and c.1115_1145del (p.D373fs*47). ET, essential thrombocythemia; PMF, primary myelofibrosis; PV, polycythemia vera; MF, myelofibrosis.
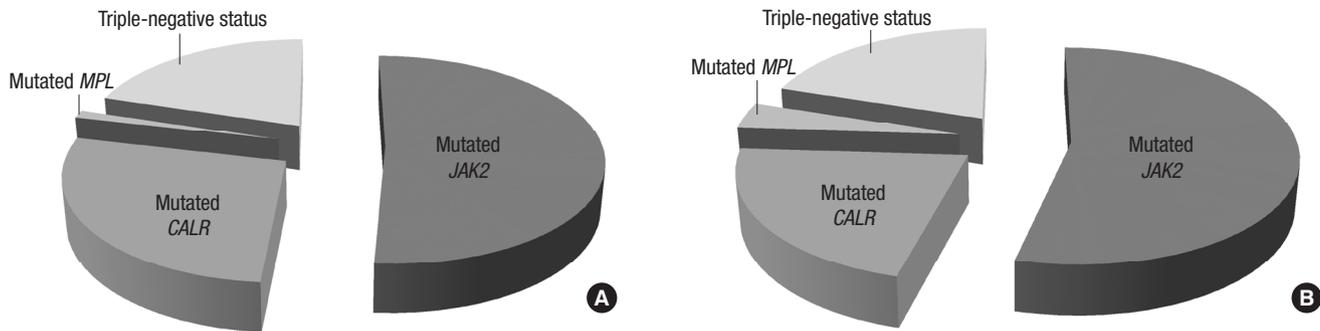
Fig. 1. Distribution of *JAK2* V617F, *MPL*, and *CALR* mutations in Korean patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF). (**A**) In patients with ET, 51.2% harbored *JAK2* V617F and 27.4% had mutations in *CALR*, and 1.2% had mutations in *MPL*, and 20.2% were negative for all 3 mutations (triple-negative). (**B**) In patients with PMF, 54.0% harbored *JAK2* V617F, 22.0% had *CALR* mutations, 4.0% had a mutation in *MPL*, and 20.0% were triple-negative.

Table 2. Clinical and laboratory features of Korean patients with essential thrombocythemia, stratified by the presence or absence of *JAK2* V617F, *MPL*, and *CALR* mutations*

Variables	*CALR* mutations (n = 23) (A)	*JAK2* V617F mutation (n = 43) (B)	Triple-negative (n = 17) (C)	*P* value		
				(A) vs. (B)	(A) vs. (C)	(B) vs. (C)
Males (%)	11 (47.8)	20 (46.5)	5 (29.4)	0.562	0.332	0.260
Age in years, median (range)	55 (19-84)	59 (26-90)	46 (22-73)	0.531	0.428	0.135
Leukocyte, ×10^9/L, median (range)	8.4 (5.3-17.8)	9.5 (3.0-40.9)	9.9 (4.3-29.4)	**0.035**	**0.039**	0.611
Hemoglobin, g/dL, median (range)	13.4 (7.0-16.3)	14.5 (7.5-18.4)	12.4 (8.1-16.4)	0.105	0.404	**0.021**
Platelet, ×10^9/L, median (range)	931 (546-1,516)	651 (453-2,435)	761 (485-2,271)	**0.006**	0.299	0.536
LD, IU/L, median (ragne)	281 (189-441)	240 (98-590)	196.5 (103-762)	0.072	**0.004**	**0.025**
Cytogenetic abnormality (%)	4/14 (28.6)	1/37 (2.7)	3/13 (23.1)	**0.017**	1.000	0.049
Thromboembolic events (%)	1 (4.3)	7 (16.3)	1 (5.9)	0.154	1.000	0.420

P values with statistically significance are shown in bold type. *A single patient harboring *MPL* W515 mutation was excluded from the analysis. LD, lactate dehydrogenase.

Table 3. Clinical and laboratory features of Korean patients with primary myelofibrosis, stratified by the presence or absence of *JAK2* V617F, *MPL*, and *CALR* mutations*

Variables	*CALR* mutations (n = 11) (A)	*JAK2* V617F mutation (n = 27) (B)	Triple-negative (n = 10) (C)	*P* value		
				(A) vs. (B)	(A) vs. (C)	(B) vs. (C)
Males (%)	5 (45.4)	16 (59.3)	8 (80.0)	0.491	0.183	0.439
Age in years, median (range)	65 (49-77)	64 (43-78)	57 (32-80)	0.936	0.181	0.137
Leukocyte, ×10^9/L, median (range)	7.2 (2.3-81.6)	12.4 (1.1-100.2)	3.4 (0.7-559.7)	0.394	0.218	0.101
Hemoglobin, g/dL, median (range)	9.5 (6.4-13.8)	11.2 (7.8-15.3)	8.1 (4.7-10.9)	0.171	**0.038**	**0.0001**
Platelet, ×10^9/L, median (range)	410 (47-1351)	216 (7-963)	90 (22-618)	**0.035**	**0.020**	0.383
Circulating blast, %, median (range)	0 (0-8)	1 (0-8)	0 (0-6)	0.463	0.876	0.639
LD, IU/L, median (range)	563 (256-916)	523 (231-2,000)	313 (191-1,976)	0.666	0.057	**0.037**
Cytogenetic abnormality (%)	2/9 (22.2)	16/26 (61.5)	6/9 (66.7)	0.059	0.153	1.000
Unfavorable karyotype (%)	2/9 (22.2)	7/26 (26.9)	4/9 (44.4)	1.000	0.620	0.416
DIPSS score, Int-2 or High (%)	5 (45.5)	8 (29.6)	6 (60.0)	0.351	0.670	0.132
Leukemic transformation (%)	1 (9.1)	3 (11.1)	3 (30.0)	0.667	0.311	0.313

P values with statistically significance are shown in bold type. *Two patients harboring *MPL* W515 mutation was excluded from the analysis. LD, lactate dehydrogenase; DIPSS, Dynamic International Prognostic Scoring System; Int, intermediate.

V617F mutation, 11 (22.0%) had *CALR* mutations, and 2 (4.0%) harbored the *MPL* W515 mutations. Ten (20.0%) patients were negative for all 3 mutations (Fig. 1B). PMF patients with *CALR* mutations accounted for 52.4% of the patients with nonmutated *JAK2* and *MPL*. Of the patients with *CALR* mutations, 9 (81.8%) patients had type 1 mutations, 1 (9.1%) patient had a type 2 mutation, and 1 (9.1%) patient had another type of mutation (Table 1).

Of the 7 patients with post-ET or PV myelofibrosis, 5 (71.4%) harbored the *JAK2* V617F mutation and the remaining 2 (28.6%) had *CALR* mutations. The 2 *CALR* mutations were only found in patients with post-ET myelofibrosis (Table 1), whereas the 2 patients with post-PV myelofibrosis who were included in this study had the *JAK2* V617F mutation. These 3 mutations (*CALR/ JAK2/MPL*) were mutually exclusive and were never detected in patients with other myeloid neoplasms with thrombocytosis.

Clinical, hematologic and molecular correlates
Tables 2 and 3 summarize the presenting clinical characteristics of study patients stratified by mutational status as follows: mutated *CALR, JAK2, MPL* and triple-negative. Univariate analysis
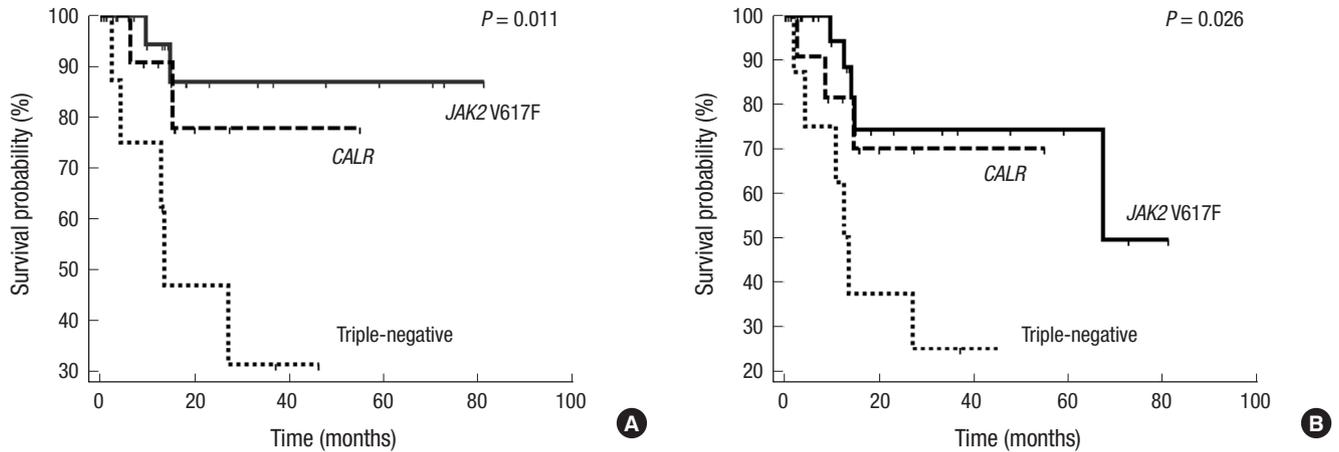
Fig. 2. Kaplan-Meier estimate of overall survival (**A**) and leukemia-free survival (**B**) of Korean patients with primary myelofibrosis according to mutational status. Two patients harboring *MPL* W515 mutations were excluded from the analysis.

of patients with ET found that *CALR* mutations were associated with a higher platelet count ($P = 0.006$), lower leukocyte count ($P = 0.035$) and higher frequency of cytogenetic abnormalities ($P = 0.017$), compared to the *JAK2* V617F mutation. *CALR* mutations were associated with a higher lactate dehydrogenase (LD) level ($P = 0.004$) and lower leukocyte count ($P = 0.039$) compared with triple-negative status. A triple-negative status was associated with a lower hemoglobin level ($P = 0.021$), lower LD level ($P = 0.025$) and higher frequency of cytogenetic abnormalities ($P = 0.049$), compared with the *JAK2* mutation (Table 2). Thromboembolic events seemed to be more frequent in patients with the *JAK2* V617F mutation compared to patients with *CALR* mutation or triple-negative status, although statistical significance was not achieved. Neither post-ET myelofibrosis nor leukemic transformation was documented during follow-up.

Of patients with PMF, *CALR* mutations were associated with a higher platelet count ($P = 0.035$), and there was a trend toward lower frequency of cytogenetic abnormalities ($P = 0.053$), compared to the *JAK2* V617F mutation. *CALR* mutations were also associated with a higher platelet count ($P = 0.020$) compared with triple-negative status. A triple-negative status was associated with a lower hemoglobin level ($P = 0.001$) and lower LD level ($P = 0.037$), compared to the *JAK2* V617F mutation. *CALR* mutations had lower frequency of leukemic transformation compared to the *JAK2* V617F mutation or triple-negative status, although statistical significance was not achieved (Table 3).

Prognostic impacts of mutations

The median follow-ups of the study population were 29.8 months (range, 0.2-120.2 months) for ET patients and 13.7 months (range, 0.2-81.2 months) for PMF patients. Four (4.8%) ET patients died, and 9 (18.0%) PMF patients died. The mortality rates of PMF patients with the *JAK2* V617F mutation, *CALR* mutations, and triple-negative status were 7.4% (2/27), 18.2% (2/11), and 50.0% (5/10), respectively.

OS and LFS times were analyzed according to the various parameters, including mutational status. Mutational status, including *CALR* and *JAK2* V617F mutations, had no significant prognostic impact on patients with ET ($P = 0.124$). However, among PMF patients, the median OS time of those with *CALR* mutations, the *JAK2* V617F mutation, and triple-negative status was 15.7, 14.3, and 13.0 months, respectively. Univariate analysis found significant survival differences between patients with *JAK2* and *CALR* mutations and triple-negative patients ($P = 0.011$ for OS and $P = 0.026$ for LFS). Triple-negative status was associated with shorter OS than *JAK2* V617F mutations (hazards ratio [HR], 7.7; 95% confidence interval [CI], 1.4-42.9) and *CALR* mutations (HR, 3.6; 95% CI, 0.8-16.0; Fig. 2A). Triple-negative status was also associated with shorter LFS than *JAK2* V617F mutations (HR, 4.3; 95% CI, 1.0-17.7) and *CALR* mutations (HR, 2.9; 95% CI, 0.8-10.8; Fig. 2B). Among the other clinical parameters, univariate analysis found that an intermediate-2 or high DIPSS score was associated with significantly shorter OS (HR, 10.8; 95% CI, 2.4-49.5; $P = 0.0001$) and LFS (HR, 6.5; 95% CI, 1.8-23.1; $P = 0.0001$) than an intermediate-1 or low score. An unfavorable karyotype was associated with shorter LFS (HR, 4.0; 95% CI, 1.1-14.7; $P = 0.005$), but not shorter OS.

By multivariate analysis, triple-negative status (HR, 7.0; 95% CI, 1.6-31.1, $P = 0.01$) and intermediate-2 or high DIPSS score (HR, 22.4; 95% CI, 2.6-196.0, $P = 0.005$) were associated with shorter OS times. Triple-negative status (HR, 6.3; 95% CI, 1.8-22.0, $P = 0.004$), intermediate-2 or high DIPSS score (HR, 9.7; 95% CI, 2.5-37.3, $P = 0.001$), and unfavorable karyotypes (HR, 4.5; 95% CI, 1.4-14.6, $P = 0.013$) were associated with shorter LFS.

DISCUSSION

The most consistent finding of this study was that among ET and PMF patients, those with *CALR* mutations had significantly higher platelet counts than those with the *JAK2* V617F mutation.

Previous studies revealed that *CALR* mutations were not found in patients with PV (1, 2), and that *CALR* mutations were found in few, if any, patients with myeloid neoplasm with thombocytosis (1). Both these observations and our results are consistent with a recent study that used measurements of the levels of calreticulin RNA and immunostaining by an antibody specific for mutated *CALR*. It found that calreticulin expression is restricted to megakaryocytes (14). In the endoplasmic reticulum (ER), calreticulin has important functions in directing the proper conformation of proteins and glycoproteins, as well as in homeostatic control of cytosolic and ER calcium levels (9-11). Because transient ER stress activation is thought to trigger the apoptotic-like phase of the thrombopoiesis process (11), the effects of calreticulin as a calcium-buffering chaperone of the ER and ultimately as a contributor to platelet production might vary, depending on the presence or absence of a *CALR* mutation. The key region during the molecular pathogenesis of Ph⁻MPN is the acidic C-domain that terminates in a KDEL sequence, and which is involved in the homeostasis of cellular calcium and is lost in mutated *CALR*. The absence of this sequence in mutated *CALR* results in altered intracellular localization and impairment of the calcium-binding function (1, 2, 15).

Overall, the other findings of our study also confirm the results of previous studies. Our study found that the frequency of *CALR* mutations was 27.4% in patients with ET and 22.0% in patients with PMF. The reported frequencies of *CALR* mutations range from 15.5% to 33% in ET patients and from 22.7% to 25% in PMF patients (3-8). The lower leukocyte counts in the *CALR*-mutated ET patients in this study, compared to the *JAK2*-mutated ET patients, has also been reported in previous studies (3, 4, 7, 8). Among patients with PMF, the triple-negative patients in our study had lower hemoglobin levels and trended toward higher prevalence of cytogenetic abnormalities and acute leukemic transformation than the *JAK2*- and *CALR*-mutated PMF patients. Ultimately, the triple-negative PMF patients had the shortest OS and LFS. These findings for triple-negative patients have also been reported by previous studies, and corroborate the adverse effects that triple-negative mutations had on the survival times of patients with PMF in our study (5, 6, 8). It is not surprising, therefore, that Tefferi et al. have emphasized that triple-negative PMF should be thought of a molecularly high-risk disease (5).

Molecular variations resulting from genetic alterations may be highly diverse between different ethnicities. Since the studies referred to in our discussion were principally performed in Western countries, a review of studies on Asian populations is needed. We found 4 very recent reports in the PubMed database on *CALR* mutations in studies of Chinese populations (16-19). On the whole, the results of these Chinese studies are in line with the results of the Western studies, regarding the frequencies of mutations and associated clinical characteristics and prognos-

tic impacts. Of note, the results of a single study on Chinese PMF patients reported by Li et al. indicate that *CALR* mutations may have different effects in different populations (19). First, their study found that 43.8% of patients with no detectable mutations in *JAK2* or *MPL* had *CALR* mutations. This frequency is substantially lower than the frequencies for PMF patients of predominantly European descent, which range from 69.4% to 74.2% (5, 6, 8), but is similar to the frequency in our study (52.4% of *JAK2*- or *MPL*-unmutated PMF patients). The reasons accounting for the discrepant findings include the different characteristics of the study patients, different sensitivities of the methods used, and ethnicity-based differences in genetic profiles. The last reason may be a reasonable explanation, because of the slightly lower frequencies of *JAK2* V617F and *MPL* mutations in Asian patients compared to Western patients. The frequencies of the *JAK2* V617F mutation in a Chinese study (19) and our study were 50% and 54%, respectively, versus 58% and 64.7% in Western studies (5, 6). The frequency of *MPL* mutations in a Chinese study (19) and our study was 3% and 4%, respectively, versus 8.3% and 7% in Western studies (5, 8). Second, the ratio between type 1 and type 2 *CALR* mutations was reversed in the Chinese PMF patients (32% were type 1 and 64% were type 2 mutations) compared with PMF patients of predominantly European descent (19). A large study of Italian PMF patients found frequencies of type 1 and type 2 mutations of 72% and 16%, respectively (6). Our results also found a predominance of type 1 mutation in PMF patients compared with ET patients. Therefore, the predominance of the *CALR* type 2 mutation in Chinese PMF patients should be confirmed in a larger Asian cohort.

The identification of disease-specific mutations justifies, in certain cases, the use of mutation analysis for determining the diagnosis, prognosis and assessment of response to treatment. *CALR* mutations are frequent in *JAK2*/*MPL*-unmutated ET/PMF and therefore are important clonal markers for such cases. Therefore, Tefferi et al. have urged that these mutations should be included as clonal markers, along with *JAK2* and *MPL* mutations, in the list of major diagnostic criteria for ET and PMF in the upcoming WHO classification system (20).

The major limitations of this study include the relatively low numbers of patients in our cohort and relatively short follow-up period. This might be major causes for the lack of data demonstrating the clinical relevance of *CALR* mutations, such as longer thromboembolic event-free survival and lower rate of thrombotic events for ET patients with *CALR* mutations than for patients with *JAK2* V617F mutations (3, 4, 8), or better OS for PMF patients with *CALR* mutations compared to PMF patients with *JAK2* V617F mutations (5, 6, 8). In addition, type 1 and type 2 *CALR* mutations have manifested as distinctly different clinical phenotypes. The type 1 mutation was found to cause in vitro cytokine-independent growth as a result of its activation of the signal transducer and activator of transcription 5, while the type

2 mutation was found to be associated with increased platelet counts and probably has an important role in the maturation of megakaryocytes (1, 7). Furthermore, in the previously mentioned study on Chinese PMF patients, the unfavorable prognostic impact of *CALR* mutations was limited to patients with type 2 mutations, and type 2 mutations or no detectable mutation was an independent unfavorable prognostic factor by multivariate analysis (19). In our study, we could not detect differences between the 2 types of *CALR* mutations, mainly because of the relatively small number of study patients.

Although a larger study cohort is definitely needed to validate the results of this study, our results should not be underestimated because, to the best of our knowledge, this is the first study that has investigated the frequency of *CALR/JAK2/MPL* mutations and related biologic and clinical features in Korean Ph⁻MPN patients. Recently, two Korean studies on *CALR* mutations were reported. However, the clinical usefulness of these studies seems to be limited, either by lack of prognostic data (21) or exclusion of *MPL* mutation and small size of each subgroup (22). Moreover, *CALR* mutations will probably be included in the major criteria for ET and PMF of the new WHO classification system (20). We have confirmed that *CALR* mutations occur in Korean patients with Ph⁻MPN that lack *JAK2* V617F or *MPL* mutations. Therefore, diagnosis of ET or PMF can now be facilitated by analyzing *CALR* mutations in Korean patients. In addition to the diagnostic value of mutation profiling, it is also useful for detecting high-risk PMF patients who are triple-negative for mutations.

In conclusion, we demonstrated that *CALR* mutations characterize a subset of Korean Ph⁻MPN patients who have distinct phenotypes similar to those reported for patients in Western countries. Because molecular markers are still unknown for approximately 50% of *JAK2*- and *MPL*-unmutated cases, morphological examination of bone marrow remains the cornerstone for accurate diagnosis for a substantial number of Korean patients suspected of having Ph⁻MPN. *CALR* mutations provide additional information on clinical outcomes, especially for patients with PMF.

DISCLOSURE

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Conceived and designed the study: Cho YU. Prepared the data: Kim BH, Bae MH. Analyzed the data: Kim BH, Bae MH, Cho YU. Wrote the first draft of the manuscript: Kim BH. Wrote the paper: Kim BH, Cho YU. Performed molecular analysis: Park YM, Lee JK, Cho YU. Contributed to the interpretation of the data and critically reviewed the draft: Jang S, Park CJ, Seo EJ, Chi HS, Choi Y, Kim DY, Lee JH, Lee JH, Lee KH. Manuscript approval: all authors.

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