# *JAK2*V617F mutation status and allele burden in classical Ph-negative myeloproliferative neoplasms in Japan

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Running head: ***JAK2*V617F allele burden in Japanese MPN**

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**Abstract**

JAK2V617F, a gain-of-function mutation in the tyrosine kinase JAK2, is frequently detected in classical myeloproliferative neoplasms (MPNs). In this study, we determined the *JAK2*V617F allele burden in Japanese MPN patients using alternately binding probe competitive-polymerase chain reaction (ABC-PCR), a highly quantitative method, which we recently developed. Although we observed strong similarities in terms of epidemiological parameters associated with the *JAK2*V617F allele burden between our cohort and others, we found a higher *JAK2*V617F allele burden in Japanese polycythemia vera (PV) patients and lower frequencies of thrombosis in Japanese MPN patients compared with previous reports. In addition, despite the presence of high red blood cell (RBC) counts, some patients bearing the *JAK2*V617F mutation were not diagnosed as PV because their hemoglobin values were lower than the WHO PV criterion. In these patients, the *JAK2*V617F allele burden was strikingly similar to that in PV patients fulfilling the 2008 WHO criteria, suggesting that these patients can be classified as PV. Although isotopic measurement of red cell mass (RCM) is required for definitive diagnosis of PV, our data imply that precise measurement of the *JAK2*V617F allele burden may improve the diagnosis of PV when RCM is not determined.

**Key words:** Myeloproliferative neoplasms (MPNs), polycythemia vera (PV), masked PV, *JAK2*V617F allele burden, alternately binding probe competitive-polymerase chain reaction (ABC-PCR)

## Introduction

JAK2V617F, a gain-of-function mutation in the tyrosine kinase JAK2, is found in the majority of patients with Ph-negative classical myeloproliferative neoplasms (MPNs) (1, 2). MPNs are subclassified as polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), with the *JAK2*V617F mutation present in approximately 95%, 50%, and 50% of cases, respectively (3-10). In murine models, the expression of *JAK2*V617F induces MPN-like phenotypes, and this variant can thus be defined as a driver mutation (11-16). The discovery of *JAK2*V617F and its prevalence in MPNs substantially contributed to our understanding of MPN pathogenesis, and thus, "presence of *JAK2*V617F" was added to the 2008 World Health Organization (WHO) diagnostic criteria for PV, ET, and PMF (17).

Although published data are somewhat inconsistent, a higher *JAK2*V617F allele burden is thought to be associated with altered hematologic parameters in PV and ET, such as higher white blood cell counts, higher hemoglobin values, and lower platelet counts (18-20). A higher *JAK2*V617F allele burden is also associated with a higher risk of thrombosis with PV and ET (19, 21, 22), a greater likelihood of developing secondary myelofibrosis with PV (23), increased splenomegaly with PV (19, 24), and increased survival with PMF (25). Taken together, the evidence for the role of *JAK2*V617F in MPNs suggests that the determination of the *JAK2*V617F allele burden has significant potential for the improvement of MPN diagnosis and patient treatment.

The epidemiologic data discussed thus far were primarily obtained from white populations, with only limited studies available from Asian populations (26-28). We previously developed a novel method, alternately binding probe competitive PCR (ABC-PCR) (29), which determines the *JAK2*V617F allele burden more accurately than previous methods such as AS-qPCR (allele-specific qPCR). In the current study, we used this new method to perform a large-scale epidemiologic study in Japan of classical MPN cases. We also examined the *JAK2*V617F allele burden and other hematologic parameters in patients who were suspected to have PV but did not fulfill the 2008 WHO criteria.

## Materials and Methods

### Sample collection and preparation

This study involved 860 individuals with suspected MPNs from the Department of Hematology at Juntendo University School of Medicine or other participating institutions in Japan between April 2010 and April 2013. If patients had been previously diagnosed with MPN and analyzed for the presence of a *JAK2* mutation, laboratory data from the diagnosis were used. This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee at Juntendo University School of Medicine (IRB#21076). Written informed consent for the use of samples and clinical records was obtained from all patients prior to sample collection. Genomic DNA was isolated from peripheral blood using a QIAamp DNA Mini Kit (Qiagen). The DNA concentration was determined using a NanoDrop LITE spectrophotometer (Thermo Scientific), and the samples were stored at -80°C until use.

### Diagnosis

Patients were diagnosed according to the 2008 WHO criteria with available indexes. We set an erythropoietin (EPO) concentration of <12.5 mU/mL as a minor criterion for PV diagnosis. For some cases in which data were obtained from a large-scale multi-center study, some indices were missing. Thus, we modified the 2008 WHO criteria as follows: 1) when bone marrow biopsies were unavailable, the bone marrow aspiration specimen, if available, was used to diagnose ET; and 2) when Bcr-Abl was not tested, the karyotype t(9;22), if available, was used to exclude chronic myelogenous leukemia.

### Endogenous erythroid colony (EEC) formation and the determination of *JAK2* exon 12 mutation

Mononuclear cells (MNCs) were separated from 20 mL of peripheral blood using Lympho separation medium (MP Biomedicals). Then, $2 \times 10^5$ MNCs were seeded in 1 mL of methylcellulose-based media (Stem Cell Technologies #H4533) and cultured at 37°C in a humidified 5% $CO_2$ atmosphere for 10 days. EEC formation was examined using

microscopy, and 10 clones for each patient were lifted for PCR analysis. A 292-base-pair DNA fragment containing the entire exon 12 of *JAK2* was PCR-amplified directly from EEC using KOD plus Neo (TOYOBO). The following primers were used for PCR: forward, 5'-GAACACATTTCATTTTACTCCTCTTTGGAG-3', and reverse, 5'-GTCACATGAATGTAAATCAAGAAAACAGAT-3'. PCR products were Sanger sequenced using the same primers.

**Detection of *JAK2* mutation and determination of *JAK2*V617F allele burden**

*JAK2*V617F presence and allele burden were determined using ABC-PCR, as described previously (29). Briefly, 1 μL of genomic DNA (10–100 ng) was used for PCR (Titanium Taq PCR kit, Takara) with primers and a fluorescence-conjugated AB-probe. A 50-cycle PCR was performed using a CFX-96 real-time PCR system (BioRad). Allele burden was determined from fluorescence intensities measured at 95°C and 55°C based on a standard curve plotted from the control. Although the ABC-PCR method generally determines the *JAK2*V617F allele burden more accurately than other methods, such as AS-qPCR, the determination of allele burdens below 10% is less accurate than with AS-qPCR. Thus, if the allele burden of a sample was below 10% with ABC-PCR, AS-qPCR was performed. For AS-qPCR, 1 μL of genomic DNA was mixed with a set of primers, TaqMan probe, and Universal PCR Master Mix (Applied Biosystems), and 50 cycles of PCR reaction were performed as previously described (30). Allele burdens below 1% as determined by AS-qPCR were considered negative for the presence of the *JAK2*V617F mutation.

**Statistics**

Levels of significance for comparison between the *JAK2*V617F allele burdens of each cohort were examined using Student's t-tests. *P* values for each data set were calculated and compared. The significance of *JAK2*V617F positivity between ET patients aged older and younger than 50 years was determined by Fisher's exact test. Correlations between *JAK2*V617F allele burden and clinical parameters (white blood cell count, hemoglobin

value, and platelet count) were determined using Pearson's product-moment correlation. *P* values below 0.05 were considered significant, and *p* values below 0.001 were indicated as <0.001.

## Results and Discussion

### Patient characteristics

Using the 2008 WHO criteria, we diagnosed 66 PV, 112 ET, and 23 PMF patients with mean ages of 66.4, 57.2, and 66.3 years, respectively (Table 1). Splenomegaly was observed in 34%, 9%, and 77% of PV, ET, and PMF patients, respectively. These frequencies are similar to a 1993–2003 Japanese study (28.8% for PV and 10.8% for ET) conducted prior to the discovery of *JAK2* mutations and their links to MPNs (31), whereas these frequencies are slightly lower than those observed in reports from Western countries (45% for PV and 19% for ET) (32, 33). Thrombotic events, including myocardial infarction, cerebral infarction, and deep vein thrombosis, were observed in 19%, 15%, and 14% patients with PV, ET, and PMF, respectively (Table 1). These frequencies are again similar to those observed in the previous Japanese study (15.4% for PV and 8.5% for ET) but are substantially lower than those observed in Western countries for PV, but not for ET (34–39% for PV and 10–29% for ET, at diagnosis of MPNs) (34). In the general population, the thrombosis risk in the Japanese population is lower than in Western populations (35), which suggests that the discrepancy in thrombotic event frequencies between Japanese and Western MPN populations is likely due to the reflection of general thrombotic event risk.

### Frequencies of the *JAK2*V617F mutation in Japanese MPN patients

To examine the *JAK2*V617F mutation and determine the allele burden of the mutant gene, we performed ABC-PCR for all MPN samples and subsequently performed AS-qPCR for those samples with allele burdens below 10% by ABC-PCR (see Materials and Methods). In total, 62 PV patients (94.0%), 63 ET patients (56.3%), and 11 PMF patients (47.8%) were positive for *JAK2*V617F (Figure 1A, Table 2), consistent with

previous reports (see Introduction).

Because we were unable to determine the presence of the *JAK2*V617F mutation in four PV patients, we obtained EEC (see Materials and Methods) from three of the four patients and performed direct sequencing. Each of the three patients exhibited a different mutation within exon 12 of *JAK2*, all of which have been reported previously (36): N542-E543del (1623_1628del); E543-D544del (1626_1631del); and K539L (1615_1616AA>TT). Although the number was limited, *JAK2* exon 12 mutations were found in PV patients who were younger than *JAK2*V617F-positive PV patients (Figure 2, Table 2), as previously described (37). Although the final patient failed to produce EEC or demonstrate the *JAK2* mutation, we observed an elevated hemoglobin value (19.5 g/dL), trilineage myeloproliferation in the bone marrow, and a low level of serum EPO, which fulfilled the 2008 WHO PV criteria. Collectively, almost 100% of the Japanese PV patients possessed mutations in *JAK2* (Figure 1A, Table 1).

In ET patients, the presence of the *JAK2*V617F mutation was associated with older onset age ($p$=0.018), higher leukocyte count ($p$=0.016), and lower platelet count ($p$=0.019), in accordance with previous studies (20) (Table 2). However, contrary to previous results, we did not observe significant differences in hemoglobin or hematocrit values between *JAK2*V617F-positive and *JAK2*V617F-negative ET patients ($p$=0.393 and $p$=0.368, respectively). For PMF, although the number of cases was limited, the presence of *JAK2*V617F was associated with older onset age ($p$=0.044) (Table 2).

**Clinical relevance of the *JAK2*V617F allele burden in Japanese MPN patients**

We determined the mean *JAK2*V617F allele burden to be 71.7%, 35.5%, and 60.8% in PV, ET, and PMF patients, respectively (Figure 1B, Table 2). The overall allele burden in our cohort, especially in PV patients, was higher than most of the published data from Western countries (19, 20, 33, 38, 39). Moreover, there were statistically significant differences in the *JAK2*V617F allele burden between PV and ET ($p$<0.001) and between ET and PMF patients ($p$=0.003), respectively, but not between PV and PMF patients ($p$=0.156) (Figure 1B). As shown in Figure 3, a higher JAK2V617F allele burden

was associated with higher hemoglobin values and lower platelet counts in ET patients ($p$=0.042 and $p$=0.049, respectively) and higher white blood cell counts in PV and ET patients ($p$<0.001 in both instances).

**Comparison of JAK2 alleles by age and gender in ET patients**

In ET, we examined *JAK2* alleles in age groups older and younger than 50 years and found that positivity for *JAK2*V617F was significantly lower in the younger population when only males were considered and when both sexes were considered (M; 15.4% vs. 63.9% $p$=0.004, Total; 35.1% vs. 66.7% $p$=0.003) (Figure 2), as previously described (18). However, when only females were considered, a substantial frequency of the younger population was *JAK2*V617F-positive (F; 45.8% vs. 69.2% $p$=0.111). The allele burdens between younger (25.4%) and older (37.2%) *JAK2*V617F-positive female ET patients showed no statistically significant difference ($p$=0.196). In addition, an increased number of JAK2V617F-negative female ET patients were diagnosed in their thirties, contributing to the formation of a "second peak" of ET onset in the female population (Figure 2), as previously noted (40). These observations suggest the presence of an unknown mechanism that accelerates ET development in the younger female population, which perhaps explains the higher ET frequencies in females compared with males (Table 1) (41).

**Diagnosis of *JAK2*V617F-positive patients with hemoglobin values below the PV threshold**

After diagnosing MPN patients according to the 2008 WHO criteria (male, hemoglobin >18.5 g/dL; female, hemoglobin >16.5 g/dL), we detected 40 patients who had the *JAK2*V617F mutation and presented high RBC counts (>600×$10^4$/μL for males, >550×$10^4$/μL for females) but were not diagnosed as PV because their hemoglobin values were below the WHO criterion. We tentatively defined these patients as "suspected PV". EPO values were available for 24 of these 40 patients, and all were low (<12.5 mU/mL). In 11 of these 40 patients, bone marrow specimens were available, and

we confirmed that 4 of the 11 samples showed apparent hypercellularity with erythroid proliferation, although the remaining 7 samples showed no obvious indication for ET or PMF diagnosis. We found that these 40 suspected PV patients presented significantly higher WBC and platelet counts compared with PV patients (Table 1), which is somewhat consistent with recent reports concerning the presence of masked PV (42). Although RCM determination is required to diagnose such patients as having PV, our results suggest that some of these patients can be classified as having masked PV.

Among these 40 patients with suspected PV, the mean corpuscular volume (MCV) was 74.8 fL, which was significantly lower than the values for healthy individuals (80–100 fL, reference value), *JAK2*-positive PV patients (84.5 fL, $p<0.001$), and ET patients (90.0 fL, $p<0.001$) (Figure 4A). Because excess production of RBCs induces an iron deficiency and a subsequent decrease of MCV, we extracted patients with normal ferritin values (15 to 150 ng/mL) from diagnosed PV (n=10) and suspected PV cases (n=8). Besides a difference in RBC-related parameters such as MCV, hematocrit and hemoglobin values, and RBC count, which are associated with the initial separation of these two groups by the hemoglobin value in the WHO criterion, hematologic parameters, including WBC and platelet counts, and the *JAK2*V617F allele burden were not significantly different (Table 3). These data strongly suggest that the majority of suspected PV patients with normal ferritin values correspond to diagnosed PV.

When patients below normal ferritin values (n=10 and n=9 for diagnosed and suspected PV, respectively) were compared, we observed not only the above-mentioned trend in RBC-related parameters but significantly higher platelet counts in suspected PV than diagnosed PV patients (Table 4). These data suggest that suspected PV patients with low ferritin values are likely to develop secondary thrombocytosis due to iron deficiency. This possibility can explain the difference in platelet counts between diagnosed and suspected PV, as described above (Table 1). Because ferritin values were only available for a limited number of patients in our cohort, further investigation will be required to support this conclusion.

### *JAK2*V617F allele burden measurement and its potential in PV diagnosis

The hemoglobin value used in the WHO 2008 criteria is specific for the diagnosis of PV but is likely much less sensitive than other parameters (43-45). The hematocrit value is another surrogate marker for RCM and is employed by the British Committee for Standards in Haematology (BCSH) guidelines to diagnose PV (46). When the BCSH guideline is applied to the above-mentioned 40 suspected PV patients, 11 are diagnosed as PV. In addition, PV patients bearing *JAK2* mutations who were diagnosed by WHO criteria (n=65) fulfilled the BCSH guideline criteria. These observations imply that the BCSH guidelines are more practical diagnostic criteria for PV diagnosis, although the specificity of these criteria may not be high (45).

Despite the fact that neither the hemoglobin nor the hematocrit value is as accurate as isotopic measurement of RCM, in Japan, virtually no institution performs this analysis. Therefore, an alternative strategy is required to identify PV in *JAK2*V617F-positive patients who present high RBC counts but low hemoglobin values. Notably, the plot of *JAK2*V617F allele burden for the above-mentioned 40 patients was strikingly similar to that of diagnosed PV patients (Figure 4B). This result suggests that patients bearing lower hemoglobin values than the 2008 WHO PV criterion can be classified as PV according to the presence of a high *JAK2*V617F allele burden as well as an increased RBC count and a decreased MCV. Since the JAK2V617F allele burden alone cannot clearly separate PV and ET patients (Figure 1B), further studies including a profiling of other mutations associated with MPN are required to include the JAK2 allele burden as a diagnostic parameter.

In summary, this is the first large-scale study analyzing the *JAK2*V617F allele burden in Japanese subjects, and our results indicate that the *JAK2*V617F allele burden is similar between Japanese and Western patients. Our highly accurate determination of *JAK2*V617F allele burden allowed us to show that the burdens of patients with low hemoglobin values and high RBC counts are similar to those of PV patients. Although RCM measurement is required for accurate PV diagnosis, the precise measurement of the *JAK2*V617F allele burden may improve the diagnosis of PV when RCM is not determined.

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## Competing Interests

The authors declare no competing interests.

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## Figure Legends

**Figure 1. *JAK2* mutation frequency and allele burden in MPN.** (A) The *JAK2* mutation frequency in PV (n=66), ET (n=112), and PMF (n=23) patients is shown; "positive" represents the *JAK2*V617F mutation, and "exon 12" represents mutations found at *JAK2* exon 12 (see text). (B) The *JAK2*V617F allele burden for the "positive" patients shown in (A) is presented. Boxes represent the interquartile range containing 50% of the subjects. The open symbol indicates the mean value, the horizontal line inside the box marks the median, and the bars show the upper and lower range of values. Significance values are indicated.

**Figure 2. *JAK2* mutation status in different age groups with MPN.** Solid, open, and shaded boxes represent *JAK2*V617F-negative, *JAK2*V617F-positive, and *JAK2* exon 12 patients, respectively.

**Figure 3. Correlation between *JAK2*V617F allele burden and hematologic parameters.** White blood cell count (WBC), hemoglobin value (Hb), and platelet count (Plt) for *JAK2*V617F-positive PV (n=62), ET (n=63), and PMF (n=11) patients are presented. Regression parameters and significance values are indicated.

**Figure 4. Patients with hemoglobin values below the WHO 2008 PV criterion and high RBC counts exhibited high *JAK2*V617F allele burdens similar to PV patients.** (A) The MCV of "suspected PV" (high RBC count but not diagnosed as PV by the 2008 WHO criteria due to the low hemoglobin value), JAK2V617F-positive PV, and ET patients is shown. The gray shadow shows the normal MCV range of healthy individuals. The MCV of "suspected PV" patients was lower compared with other patients. (B) The *JAK2*V617F allele burden of "suspected PV" patients was similar to that of PV patients and higher than that of ET patients. The allele burdens for PV and ET patients, as shown in Figure 1B, are depicted for "suspected PV" patients. Boxes represent the interquartile range containing 50% of the subjects, the open symbol indicates the mean value, the

481 horizontal line inside of the box marks the median, and the bars show the upper and
482 lower range of values.
483

Figure 1

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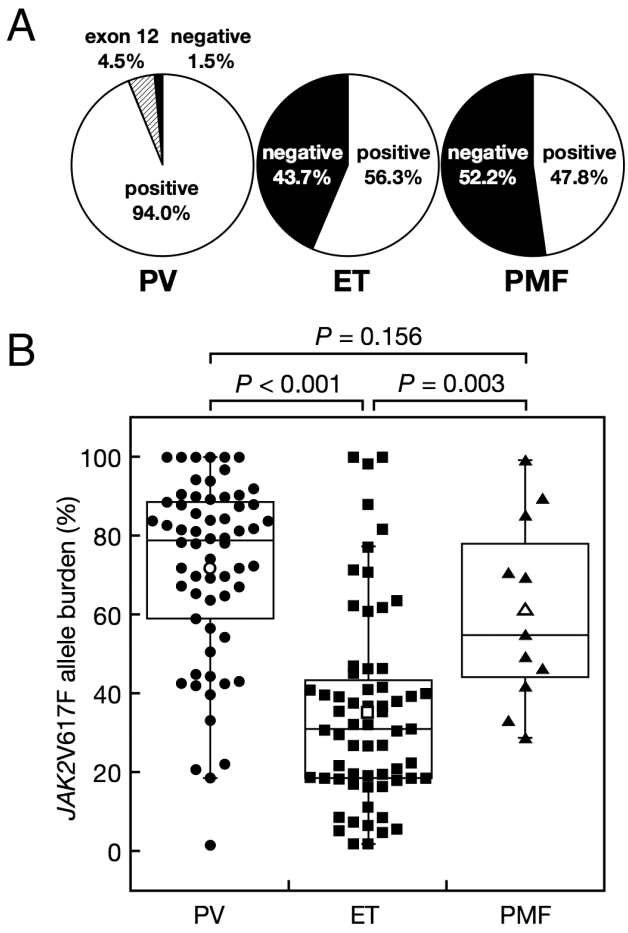

Figure 2

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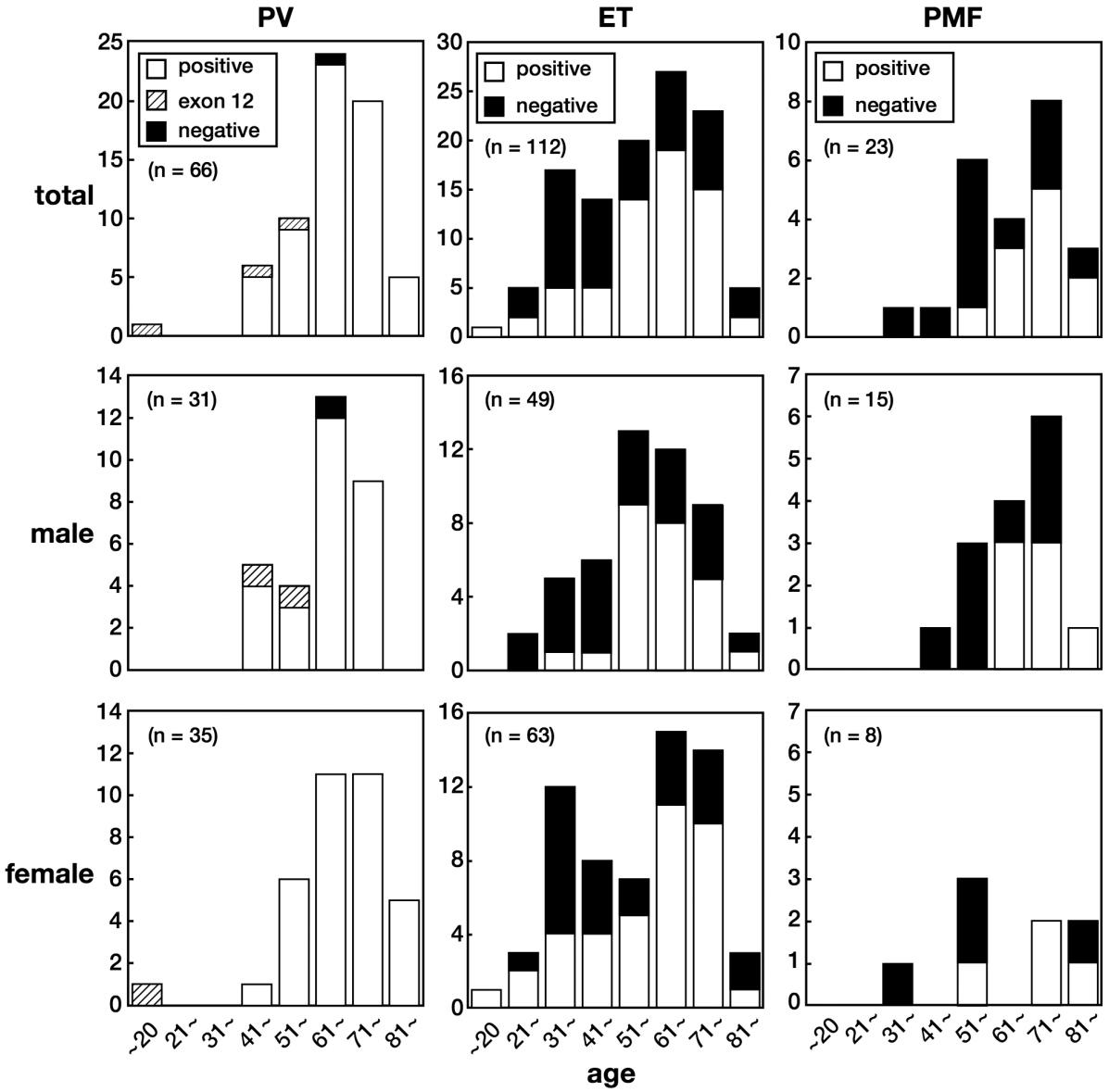

Figure 3

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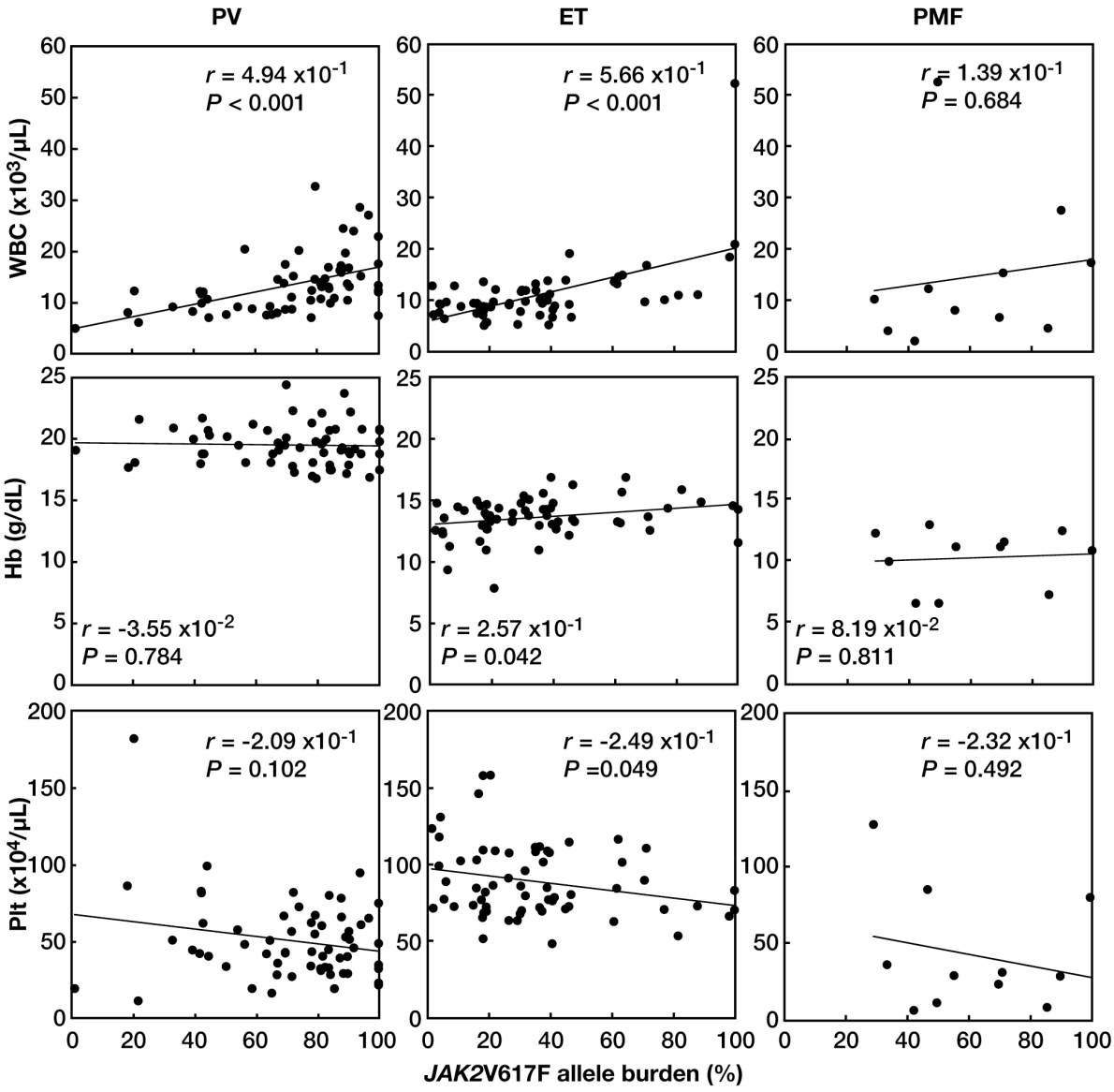

Figure 4

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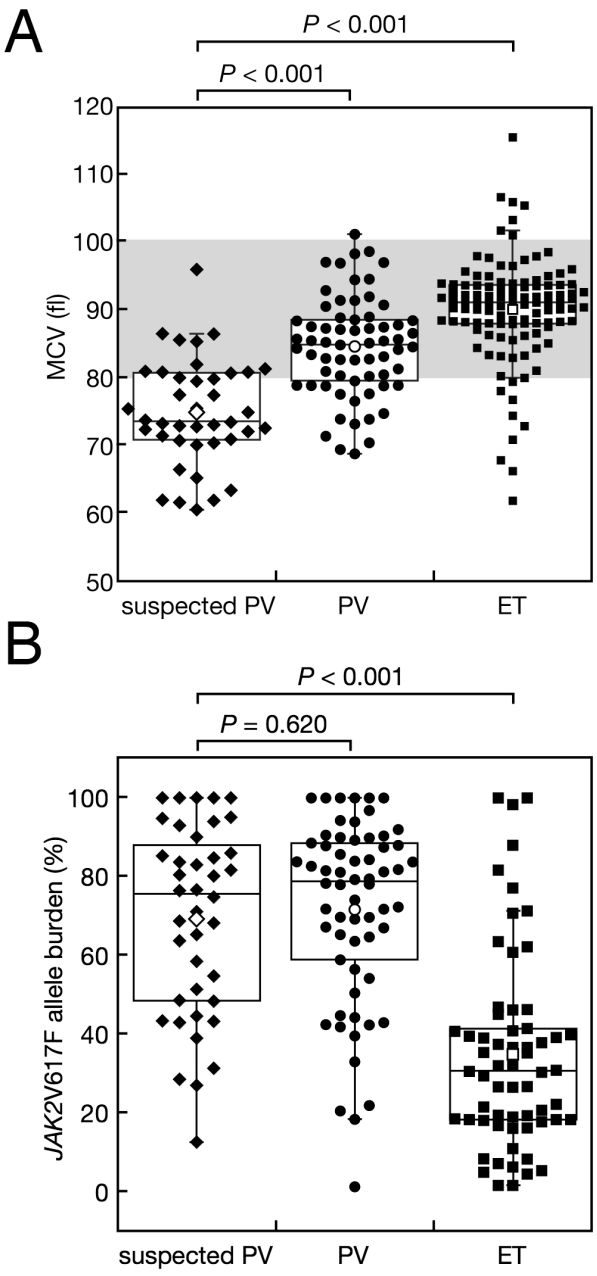

**Table 1: Clinical and hematologic characteristics at diagnosis in MPN patients from this study.**

| | PV | ET | PMF | Suspected PV | *p* value (PV vs. suspected PV) |
|---|---|---|---|---|---|
| Number of patients (M:F) | 66 (31:35) | 112 (49:63) | 23 (15:8) | 40 (17:23) | - |
| Age (years, mean±SD) | 66.4±11.9 (20–90) | 57.2±15.8 (19–86) | 66.3±12.8 (38–88) | 69.3±10.7 (45-89) | 0.194 |
| WBC ($\times 10^9$/L, mean±SD) | 13.2±5.7 (5.2–32.8) | 9.9±5.3 (4.1–52.3) | 12.3±12.8 (2.2–52.8) | 18.3±9.2 (8.6-61.8) | <0.001 |
| RBC ($\times 10^4$/μL, mean±SD) | 714.0±91.9 (548–970) | 464.8±70.2 (259–706) | 350.7±95.1 (192–576) | 644.5±60.6 (561-802) | <0.001 |
| Hct (%, mean±SD) | 59.9±5.2 (51.7–72.7) | 41.5±4.7 (25.4–54.1) | 30.5±7.2 (17.6–40.5) | 48.0±4.8 (38.5-58.6) | <0.001 |
| Hb (g/dL, mean±SD) | 19.4±1.6 (16.6–24.3) | 13.6±1.6 (7.9–17.0) | 9.7±2.3 (5.7–13.0) | 15.0±1.7 (11.7-18.5) | <0.001 |
| MCV (fL) | 84.5±7.4 (68.6-101.1) | 90.0±7.8 (61.7-115.4) | 88.1±7.6 (60.8-98.5) | 74.8±7.8 (60.4-95.9) | <0.001 |
| Platelet ($\times 10^9$/L, mean±SD) | 495.6±261.4 (114–1815) | 958.2±352.0 (474–2832) | 412.2±392.8 (2–1491) | 812.9±463.7 (138-2488) | <0.001 |
| Erythropoietin (mU/mL)* | 7.8±2.5 (n=63) (1.0–12.1) | 23.9±43.0 (n=20) (6.8-205.0) | 18.4±8.5 (n=5) (9.7–31.0) | 7.8±2.8 (n=24) (2.0-12.0) | 0.511 |
| Ferritin (ng/mL)* | 18.7±15.7 (n=20) (6.0-78.0) | 95.2±88.2 (n=36) (5.0-369.2) | 202.9±348.0 (n=5) (4.0-823.0) | 22.0±17.3 (n=17) (3.0-70.0) | 0.825 |
| Splenomegaly* | 21/61 (34%) | 10/106 (9%) | 17/22 (77%) | 15/39 (38%) | 0.844 |
| Thrombotic event* | 10/53 (19%) | 14/96 (15%) | 2/14 (14%) | 9/27 (33%) | 0.297 |
| Myelofibrosis* | 0/29 (0%) | 7/109 (6%) | 23/23 (100%) | 0/11 (0%) | 1.000 |
| *JAK2* mutation presence | 65 (98.5%) | 63 (56.3%) | 11 (47.8%) | 40 (100%) | - |

M, male; F, female; SD, standard deviation; WBC, white blood cell count; RBC, red blood cell count; Hct, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume. *A subset of patients was evaluated.

**Table 2: Clinical and hematologic characteristics according to *JAK2* mutant status**

| | PV | | | ET | | PMF | | *p* value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *JAK2* mutation | V617F (A) | exon12 (B) | negative | V617F (C) | negative (D) | V617F (E) | negative (F) | A vs. B | C vs. D | E vs. F |
| Number of patients (%) | 62 (94.0) | 3 (4.5) | 1 (1.5) | 63 (56.3) | 49 (43.7) | 11 (47.8) | 12 (52.2) | - | - | - |
| Male:Female (N) | 28:34 | 2:1 | 1:0 | 25:38 | 24:25 | 7:4 | 8:4 | - | - | - |
| Age (years, mean±SD) | 67.5±10.3 (43-90) | 41.7±19.9 (20-59) | 70 | 61.4±14.7 (19-83) | 53.2±16.6 (26-86) | 71.9±9.8 (52-88) | 61.3±13.5 (38-81) | <0.001 | 0.018 | 0.044 |
| WBC ($\times10^9$/L, mean±SD) | 13.6±5.7 (5.1-32.8) | 7.6±2.4 (5.2-10.0) | 7.5 | 11.0±6.2 (5.2-52.3) | 8.6±3.4 (4.1-21.9) | 14.8±14.6 (2.2-52.8) | 10.1±11.1 (3.2-44.1) | 0.078 | 0.016 | 0.394 |
| RBC ($\times10^4$/μL, mean±SD) | 710.9±88.4 (548-881) | 803.3±144.5 (713-970) | 640 | 474.4±80.1 (259-706) | 450.8±54.6 (344-616) | 387.5±107.9 (223-576) | 316.9±70.1 (192-411) | 0.090 | 0.081 | 0.074 |
| Hct (%, mean±SD) | 59.8±5.2 (51.7-72.7) | 60.5±7.4 (52.6-67.2) | 58.8 | 41.8±5.1 (25.4-54.1) | 40.9±4.2 (33.5-50.0) | 32.8±7.5 (20.4-40.5) | 28.5±6.5 (17.6-38.7) | 0.823 | 0.368 | 0.154 |
| Hb (g/dL, mean±SD) | 19.4±1.6 (16.7-24.3) | 19.0±2.1 (16.6-20.6) | 19.5 | 13.7±1.6 (7.9-16.9) | 13.4±1.6 (10.8-17.0) | 10.3±2.4 (6.6-13.0) | 9.1±2.2 (5.7-12.7) | 0.698 | 0.393 | 0.228 |
| MCV (fL) | 84.8±7.1 (68.6-101.1) | 76.0±8.1 (69.3-85.0) | 91.9 | 89.1±8.4 (66.1-106.6) | 91.2±6.9 (61.7-115.4) | 86.2±10.1 (60.8-98.4) | 89.8±4.1 (83.5-98.5) | 0.042 | 0.147 | 0.264 |
| Platelet ($\times10^9$/L, mean±SD) | 504.6±265.6 (114-1815) | 380.0±158.5 (286-563) | 288 | 889.7±239.5 (486-1580) | 1046.2±445.3 (474-2832) | 430.2±384.4 (70-1280) | 395.7±416.7 (2-1491) | 0.426 | 0.019 | 0.839 |
| Erythropoietin (mU/mL)* | 7.8±2.5 (1.0-12.1) (n=59) | 5.9±1.7 (4.0-7.2) (n=3) | 11.7 (n=1) | 12.2±4.6 (6.8-22.2) (n=12) | 41.5±66.2 (9.6-205) (n=8) | 18.4±8.5 (9.7-31.0) (n=5) | | 0.189 | 0.139 | |
| *JAK2*V617F allele burden (%, mean±SD) | 71.7±23.1 (1.5-100.0) | | | 35.5±24.8 (1.9-100.0) | | 60.8±23.7 (28.8-99.3) | | | | |

M, male; F, female; SD, standard deviation; WBC, white blood cell count; RBC, red blood cell count; Hct, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume. *A subset of patients was evaluated.

**Table 3: Clinical and hematologic characteristics of diagnosed and suspected PV patients with normal ferritin values**

| | PV with normal ferritin (≥15 ng/mL) | Suspected PV with normal ferritin (≥15 ng/mL) | *p* value |
|---|---|---|---|
| Number of patients (M:F) | 10 (7:3) | 8 (3:5) | - |
| Age (years, mean±SD) | 70.8±9.6 (48-82) | 63.8±12.0 (45-81) | 0.183 |
| WBC ($\times 10^9$/L, mean±SD) | 14.6±6.6 (7.2–27.2) | 24.4±17.0 (11.3-61.8) | 0.112 |
| RBC ($\times 10^4$/μL, mean±SD) | 700.0±79.7 (594–842) | 619.4±41.7 (563-694) | 0.020 |
| Hct (%, mean±SD) | 58.4±3.2 (54.3–64.9) | 47.1±3.2 (42.1-50.8) | <0.001 |
| Hb (g/dL, mean±SD) | 19.1±1.2 (16.8–20.6) | 14.4±1.2 (12.5-15.7) | <0.001 |
| MCV (fL) | 84.2±9.4 (68.6–97.0) | 76.2±4.4 (70.6–80.8) | 0.041 |
| Platelet ($\times 10^9$/L, mean±SD) | 678.2±447.5 (332–1815) | 769.8±588.9 (138-1974) | 0.712 |
| Erythropoietin (mU/mL)* | 5.9±2.5 (n=8) (1.6-9.9) | 8.4±2.9 (n=5) (4.1-11.1) | 0.136 |
| Ferritin (ng/mL)* | 27.8±18.2 (16-78) | 35.6±16.5 (17-70) | 0.358 |
| Splenomegaly* | 5/9 (56%) | 4/7 (57%) | 1.000 |
| Thrombotic event* | 2/8 (25%) | 1/4 (25%) | 1.000 |
| Myelofibrosis* | 0/7 (0%) | 0/1 (0%) | 1.000 |
| *JAK2*V617F allele burden (%, mean±SD) | 65.6±26.4 (20.7-96.8) | 79.2±29.4 (12.8-100.0) | 0.317 |

M, male; F, female; SD, standard deviation; WBC, white blood cell count; RBC, red blood cell count; Hct, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume. *A subset of patients was evaluated.

**Table 4: Clinical and hematologic characteristics of diagnosed and suspected PV patients with low ferritin values**

| | PV with low ferritin (<15 ng/mL) | Suspected PV with low ferritin (<15 ng/mL) | *p* value |
|---|---|---|---|
| Number of patients (M:F) | 10 (4:6) | 9 (5:4) | - |
| Age (years, mean±SD) | 59.9±17.0 (20-78) | 71.8±7.6(62-85) | 0.071 |
| WBC ($\times 10^9$/L, mean±SD) | 11.4±4.2 (7.2–20.3) | 14.1±3.1 (10.0-19.1) | 0.135 |
| RBC ($\times 10^4$/μL, mean±SD) | 770.4±119.4 (577–970) | 666.4±51.8 (597-754) | 0.028 |
| Hct (%, mean±SD) | 60.3±6.5 (52.3–70.5) | 46.6±6.1 (39.1-58.6) | <0.001 |
| Hb (g/dL, mean±SD) | 19.3±1.9 (16.6–22.2) | 14.4±2.3 (11.7-18.5) | <0.001 |
| MCV (fL) | 79.1±7.4 (69.3–90.6) | 69.9±7.6 (60.4–80.7) | 0.017 |
| Platelet ($\times 10^9$/L, mean±SD) | 449.2±165.5 (272–724) | 727.2±283.1 (387-1162) | 0.017 |
| Erythropoietin (mU/mL)* | 7.6±2.9 (n=9) (4.0-11.9) | 7.7±1.8 (n=5) (5.0-9.4) | 0.927 |
| Ferritin (ng/mL)* | 9.8±2.6 (6-14) | 9.8±3.3 (3-14) | 0.973 |
| Splenomegaly* | 5/10 (50%) | 4/9 (44%) | 1.000 |
| Thrombotic event* | 1/8 (13%) | 2/6 (33%) | 0.577 |
| Myelofibrosis* | 0/7 (0%) | 0/4 (0%) | 1.000 |
| *JAK2*V617F allele burden (%, mean±SD) | 74.3±17.6 (n=8**) (33.2-90.4) | 65.8±23.1 (n=9) (31.5-95.1) | 0.414 |

M, male; F, female; SD, standard deviation; WBC, white blood cell count; RBC, red blood cell count; Hct, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume. *A subset of patients was evaluated. **Two patients possessing JAK2 exon 12 were not included.