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RESEARCH LETTER

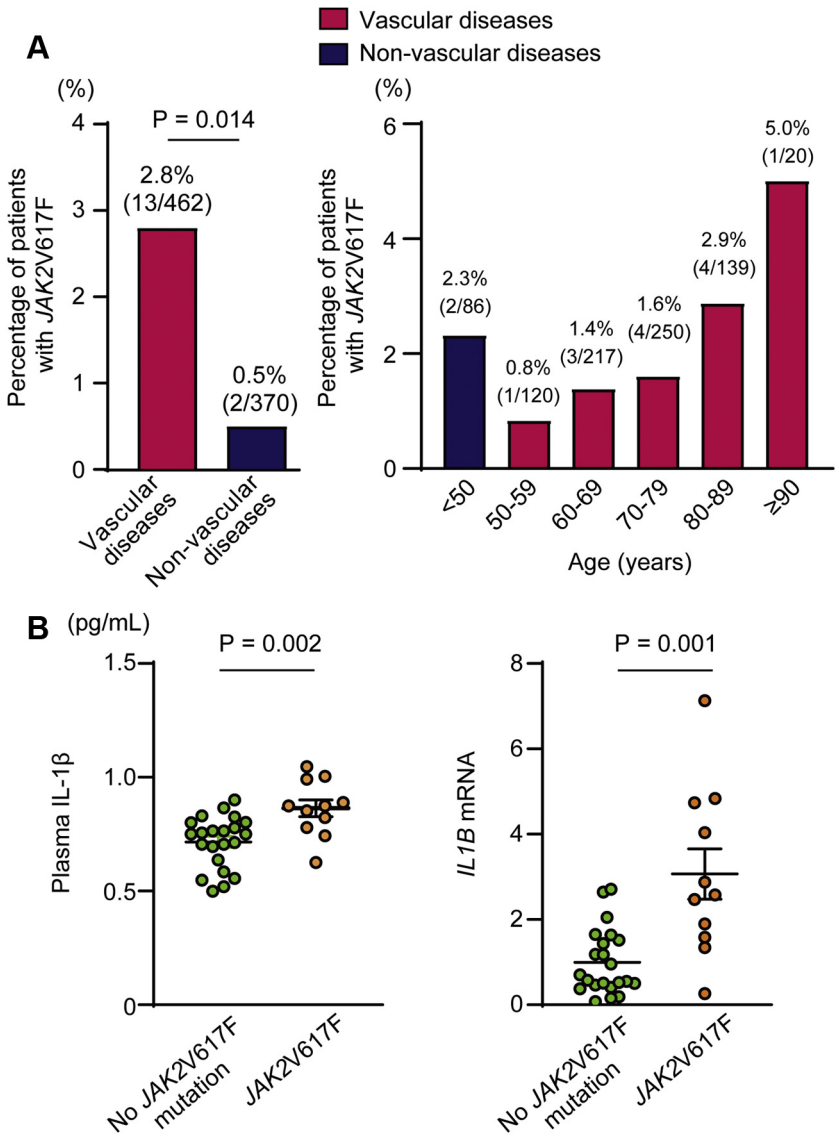
Clonal Hematopoiesis and *JAK2*V617F Mutations in Patients With Cardiovascular Disease

Mutations in *JAK2* are associated with clonal hematopoiesis (CH) (1,2). *JAK2*V617F is present in myeloproliferative neoplasms (MPNs), whereas *CALR* and *MPL* mutations have been detected in *JAK2*V617F-negative MPNs. Although these gene mutations are associated with the cytokine signaling via JAK2/STAT pathways, the prevalence and clinical significance of CH with MPN-driving mutations of low variant allele frequency (VAF) in cardiovascular disease (CVD) is not clear.

We recruited 877 consecutive hospitalized patients with CVD at Fukushima Medical University Hospital between March 2018 and November 2019. We excluded patients with hematological malignancies (n = 9) and those who did not consent (n = 36). Patients with coronary disease, peripheral arterial disease, aortic aneurysm and/or aortic dissection, ischemic stroke, and deep vein thrombosis and/or acute pulmonary thromboembolism were categorized as having vascular disease (n = 462). This subgroup was compared with those without established vascular disease (n = 370), which included patients with primary cardiomyopathy, valvular heart disease, arrhythmia, congenital heart disease, and myocarditis. In these 832 patients, we isolated genomic DNA from peripheral blood and determined the VAF of *JAK2*V617F, *CALR*del52, *CALR*ins5, *MPL*W515L, and *MPL*W515K by allele-specific quantitative polymerase chain reaction (PCR) using primers and Taqman probes specific to wild-type and mutant alleles with the delta Ct method, using appropriate validation and PCR efficiencies (3). The cutoff values of VAF were 0.04% to 0.23% for each mutation. The protocol was approved by the institutional ethical committee of Fukushima Medical University Hospital (approval number, 29348).

For these 832 patients, the mean age was 67.8 ± 13.4 years, and 68.4% were men. Older age (69.7 ± 11.9 years vs. 65.4 ± 14.7 years), diabetes (43.3% vs. 24.3%), hypertension (71.4% vs. 53.5%), and dyslipidemia (85.1% vs. 58.6%) were more prevalent in the vascular disease group than that of the nonvascular disease group. Body mass index (24.0 ± 3.8 kg/m^2 vs. 23.5 ± 3.8 kg/m^2) and tobacco use (16.5% vs. 14.1%) were similar. Sixteen patients (1.9%) exhibited MPN-driving mutations, including 15 patients with a *JAK2*V617F (1.8%) and 1 patient with a *CALR*ins5 (0.12%) mutation. No patients carried *CALR*del52, *MPL*W515L, or *MPL*W515K mutations. Because the VAFs were all <2%, the patients were not classified as CH of indeterminate potential (CHIP). The VAF of *JAK2*V617F ranged from 0.06% to 1.73% (0.45 ± 0.56%). The VAF of a *CALR*ins5-positive patient was 0.25%. None of the patients with *JAK2*V617F or *CALR*ins5 met the criteria of MPNs according to the 2016 World Health Organization classification. There were 13 *JAK2*V617F-positive patients in the vascular disease group; the prevalence of *JAK2*V617F-mediated CH was significantly higher in the vascular disease group than that of the nonvascular disease group (2.8% vs. 0.5%; p = 0.014) and increased with age (**Figure 1A**). Propensity matching that determined the prevalence of *JAK2*V617F-positive patients across disease groups demonstrated similar results. In multivariable logistic regression analysis adjusted for confounders, including age, sex, body mass index, hypertension, diabetes mellitus, dyslipidemia, and tobacco use, *JAK2*V617F was associated with vascular disease (odds ratio: 5.44; 95% confidence interval [CI]: 1.05 to 28.23; p = 0.043). Plasma interleukin (IL)-1β concentrations and leukocyte *IL1B* mRNA expressions were significantly increased in patients with vascular disease with *JAK2*V617F compared with age- and sex-matched patients with vascular disease without mutations (**Figure 1B**). During a median follow-up of 441 days (quartile 1 to quartile 3: 249 to 661 days), major cardiovascular events occurred in 61 patients, including 29 cardiac deaths, 23 unplanned rehospitalizations due to heart failure, 5 myocardial infarctions and/or revascularizations, and 4 ischemic strokes. Non-cardiovascular death occurred in 24 patients. There were 4 cardiovascular events in 15 *JAK2*V617F-positive patients (1 cardiac death, 2 revascularizations, 1 heart failure hospitalization). In considering the competing

FIGURE 1 ***JAK2*V617F-Mediated CH Is Associated With Vascular Diseases and IL-1β**



(A) The prevalence of *JAK2*V617F-mediated clonal hematopoiesis (CH) was more common in patients with vascular disease than in patients without vascular disease **(left)** and increased with age **(right)**. The numbers of *JAK2*V617F-positive patients are indicated. The statistical comparison was performed by the chi-square test. **(B)** Plasma interleukin (IL)-1β concentrations were assessed by enzyme-linked immunosorbent assay, and *IL1B* mRNA expression levels in peripheral leukocytes were assessed by quantitative reverse transcription polymerase chain reaction in patients with vascular disease with *JAK2*V617F (n = 11) in comparison to age- and sex-matched patients with vascular disease with no *JAK2*V617F mutations (n = 22). *IL1B* mRNA levels were normalized by *GAPDH* and expressed as a relative ratio over the patients with no *JAK2*V617F mutations. The comparisons were performed by the Mann-Whitney U test.

risks of non-cardiovascular death using a Fine-Gray method (n = 832) adjusted for age, sex, body mass index, hypertension, diabetes mellitus, dyslipidemia, and tobacco use, *JAK2*V617F was independently associated with a risk of cardiovascular events (subdistribution hazard ratio: 3.35; 95% CI: 1.22 to 9.21; p = 0.019).

Although *JAK2*V617F mutations are well-established in CHIP, our findings suggested that even MPN-driving mutations with a low VAF are potentially relevant to CVD, demonstrating that *JAK2*V617F-mediated CH was more common in patients with vascular disease and was associated with an increased risk of cardiovascular events. *JAK2*V617F-

CH was associated with increased IL-1β specifically in patients with vascular disease, similar to *DNMT3A*- and *TET2*-CH (4,5). Further studies are clearly needed to validate our findings in larger cohorts and elucidate the causal relationship between *JAK2*V617F and cardiovascular outcomes. These findings generate further hypotheses related to the potential relevance of *JAK2*V617F-mediated CH with low VAF in vascular disease populations.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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