JAK2 p.V617F Variants in Non-Blood DNA from Patients with Polycythemia Vera

Polistemia Vera Hastalarında Kan Kökenli Olmayan DNA'da *JAK2* p.V617F Varyantları

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To the Editor,

The p.V617F mutation of the *Janus kinase 2* (*JAK2*) gene has been identified in 95% of patients with polycythemia vera (PV) and in half of patients with essential thrombocythemia (ET) [1,2,3,4]. The majority of cases of myeloproliferative neoplasms (MPNs) are sporadic, while a familial predisposition to MPNs has been reported [5]. Germline transmission of *JAK2* p.V617F variants has not been reported in familial PV, although germline p.V617I, p.Y317H, and p.T875N variants were reported in a family with hereditary thrombocytosis or erythrocytosis [6,7]. *JAK2* single-nucleotide variant (SNV) 46/1 is associated with a weak predisposition to MPNs but the *JAK2* 46/1 haplotype does not explain familial MPNs [8].

To determine whether the *JAK2* p.V617F variant was present in the germline, we analyzed the variants of 11 patients (10 with PV and 1 with ET) including siblings (PV1 and PV2) and one patient (PV25) who had an identical twin brother with *JAK2* p.V617F-positive PV. Peripheral blood (PB), fingernail, toenail, and hair samples were collected from 11, 11, 2, and 4 patients, respectively, after obtaining written informed consent. The current study was conducted with the approval of the relevant institutional ethics committee.

To quantify the allele burden of the p.V617F variant, we conducted target sequencing with the Ion AmpliSeq Cancer Hotspot Panel v2 and Ion Proton™ using the PI Chip (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing data were analyzed with Torrent Suite software (Ver. 5.0.4; Thermo Fisher Scientific, Waltham, MA, USA). The mean coverage was 3792.2 and 100x coverage ranged from 98.32% to 100% for each sample. The allele burden of the PB samples was 9.6%-100% for 11 patients while that of the fingernail samples was above the cutoff value (3% for SNVs and indels at hotspots) for 4 of 11 patients (Table 1). The allele burden of a fingernail sample (42.3%) was close to that of the PB (45.8%) for one patient with sporadic PV (PV12). To confirm the variants, direct sequencing was performed using a 3730xL DNA Analyzer (Life Technologies, Carlsbad, CA, USA) [3]. The variants were detected in the fingernail and hair samples of PV12, and the variant intensity of the fingernail and hair samples was similar to or higher than that of the PB (Table 1, Figure 1), suggesting germline variants.

In two patients with sporadic PV (PV16 and PV18), the allele burden of the fingernail samples (6.5% and 8.1%) was lower than that of the PB (44.1% and 98.0%), and the allele burden of their different fingernails was 3.9% and 4.0%, respectively. The variants were detected by direct sequencing in the fingernail samples of PV16 and PV18, but the variant intensity of the fingernails was lower than that of the PB (Table 1, Figure 1). Since nails were favorable materials without contamination from leukocytes, these findings may represent mosaicism [9].

The *JAK2* 46/1 G allele was identified in 10 of 11 patients, including the siblings, but the identical twin did not have the G allele. To our knowledge, this is the first report on the possible germline *JAK2* p.V617F variant in patients with sporadic PV. Further studies will clarify the significance of *JAK2* p.V617F variants in non-blood samples from MPN patients.

Table 1. *JAK2* p.V617F variant in patients with polycythemia vera and essential thrombocythemia.

Case	Age	Sex	WBC, x10^9/L	RBC, x10^{12}/L	Hb, g/L	Ht %	Platelets, x10^9/L	Splenomegaly	Karyotype	SNV46/1	Allele burden-target sequencing				Direct sequencing		
											PB	Fingernail 1	Fingernail 2	Toenail	PB	Fingernail	Hair
PV1	71	M	18.7	6.26	178	55.2	722	Slight	46,XY/47,XY+9	CG	65.3	<Cut-off	<Cut-off	<Cut-off	+	~Low*	-
PV2	59	M	7.7	7.45	230	70.5	278	+	NA	CG	100	4.9	<Cut-off	<Cut-off	+	~Low*	-
PV4	61	M	6.4	7.08	181	58.5	253	Slight	NA	GG	39.0	<Cut-off			+	+	
PV9	57	F	6.8	5.57	186	53.8	432	+	46,XX	CG	49.3	<Cut-off			+	-/+	
PV11	60	F	9.7	6.64	181	55.8	508	Slight	46,XX	GG	26.4	<Cut-off			Low	-/+	
PV12	61	F	11.5	6.61	191	57.8	721	Slight	46,XX	GG	45.8	42.3			+	+	+
PV15	50	F	22.1	8.60	211	64.0	816	NA	46,XY	CG	88.0	<Cut-off			+	-	
PV16	44	M	8.1	5.45	166	48.5	776	-	46,XY	CG	44.1	6.5	3.9		+	Low*	
PV18	57	F	11.2	8.11	223	72.5	364	+	NA	CG	98.0	8.1	4.0		+	Low*/+	
PV25	27	M	12.9	6.14	177	51.4	86.7	-	46,XY	CC	50.2	<Cut-off			+	-	-
ET6	60	F	16.1	4.89	132	39.5	1270	-	46,XX	CG	9.6	<Cut-off			Low	-	

WBC: White blood cells; RBC: red blood cells; Hb: hemoglobin; Ht: hematocrit; PV: polycythemia vera; ET: essential thrombocythemia; M: male; F: female; SNV: single-nucleotide variant; PB: peripheral blood.
*Low-intensity signals were seen.

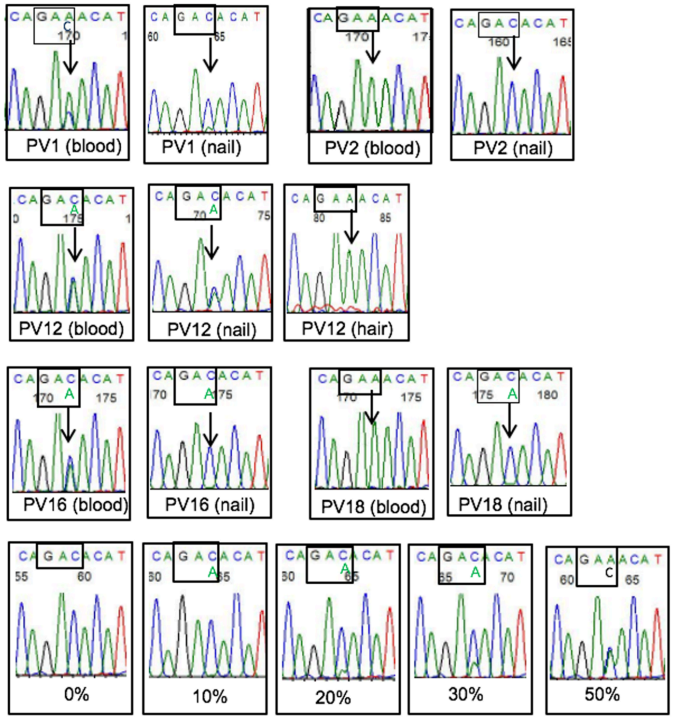

Figure 1. JAK2 p.V617F variant in polycythemia vera patients. Direct sequencing of the JAK2 gene was performed for polycythemia vera patients (antisense). To determine the sensitivity, DNA from the commercial control (50%) was diluted using DNA from the wild-type cell line to obtain the following percentages: 0%, 10%, 20%, 30%, and 50%. Sensitivity to the p.V617F variant by direct sequencing was about 10%. The JAK2 p.V617F variant was found in fingernail (PV12, PV16, PV18) and hair (PV12) DNA from polycythemia vera patients.

Keywords: Polycythemia vera, Essential thrombocythemia, *JAK2* p.V617F mutation

Anahtar Sözcükler: Polisitemi vera, Esansiyel trombositemi, *JAK2* p.V617F mutasyonu

Ethics

Ethics Committee Approval: The current study was conducted with the approval of the Tokyo Women's Medical University's Ethics Committee.

Informed Consent: Informed consent was obtained from the patients.

Authorship Contributions

Concept- N.M.; Design- K.Y., M.S., J.T.; Data Collection or Processing- M.S., R.D.; Analysis or Interpretation- N.M., K.Y., M.O.M., M.S., J.T.; Writing- N.M.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This work was supported by JSPS KAKENHI, Grant Number JP19K07447.

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Received/Geliş tarihi: April 18, 2023
Accepted/Kabul tarihi: June 6, 2023

DOI: 10.4274/tjh.galenos.2023.2023-0159