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Simultaneous screening for JAK2 and calreticulin gene mutations in myeloproliferative neoplasms with high resolution melting

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ABSTRACT

Introduction: Recently, novel calreticulin (CALR) mutations were discovered in Janus kinase 2 (JAK2) non-mutated myelofibrosis (PMF) and essential thrombocythemia (ET) cases, with a frequency of 60–80%. We examined clinical correlations and CALR mutation frequency in our myeloproliferative neoplasms (MPN) cases, and introduce an effective test method for use in clinical practice.
Methods: We examined 177 samples previously investigated for the JAK2 mutation for differential diagnosis of MPN. JAK2 and CALR mutations were analyzed using melting curve analysis and microchip electrophoresis, respectively. Next, we constructed a test for simultaneous screening of the JAK2 and CALR mutations utilizing high resolution melting (HRM).
Results: Among 99 MPN cases, 60 possessed the JAK2 mutation alone. Of the 39 MPN cases without the JAK2 mutation, 14 were positive for the CALR mutation, all of which were ET. Using our novel screening test for the JAK2 and CALR mutations by HRM, the concordance rate of conventional analysis with HRM was 96% for the JAK2 mutation and 95% for the CALR mutation.
Conclusion: Our novel simultaneous screening test for the JAK2 and CALR gene mutations with HRM is useful for diagnosis of MPN.

1. Introduction

Myeloproliferative neoplasms (MPN) are a group of chronic myeloid cancer diseases characterized by overproduction of mature blood cells [1,2]. Except for chronic myelogenous leukemia (CML), the 3 most common types of MPN are polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). In many cases, these diseases develop slowly and gradually worsen, while some can progress to leukemia. Many patients with BCR-ABL-negative MPN carry the Janus kinase 2 V617F (JAK2) mutation [3,4]. Either that or JAK2 exon 12 mutation are found in most patients with PV, whereas one of those is found in only 50–60% of patients with ET or PMF [5–7]. In addition, mutations in the thrombopoietin receptor gene (myeloproliferative leukemia, MPL) have been reported in patients with JAK2 mutation-negative MPN. MPL mutational frequencies are estimated at 3% in ET and 10% in PMF [8]. Tests for the JAK2 mutation have greatly simplified diagnosis of MPN and are now firmly established as front-line examinations [9]. However, distinguishing ET cases possessing non-mutated JAK2 from the much more common reactive thrombocytosis remains a major diagnostic problem.

In December 2013, two groups reported novel calreticulin (CALR) mutations (exon 9 deletions and insertions) in ET and PMF patients possessing non-mutated JAK2 or MPL [10,11]. In the first of those studies [10], they examined 1107 samples from patients with MPN and noted that CALR mutations were not seen in any with PV ($n = 382$), whereas they were detected in patients with ET ($n = 311$) or PMF ($n = 203$). Interestingly, the occurrence of CALR mutations was

Abbreviations: (JAK2), Janus kinase 2; (MPN), myeloproliferative neoplasms; (PV), polycythemia vera; (ET), essential thrombocythemia; (PMF), Primary myelofibrosis; (CALR), calreticulin; (HRM), high resolution melting; (CML), chronic myelogenous leukemia; (Hb), hemoglobin; (WBC), white blood cell; (PLT), platelet; (MCV), mean corpuscular volume; (MPV), mean platelet volume; (LD), lactate dehydrogenase; (IL-3), interleukin-3; (JAK-STAT), JAK-signal transducer and activator of transcription; (MDS), myelodysplastic syndrome; (AML), acute myelogenous leukemia.

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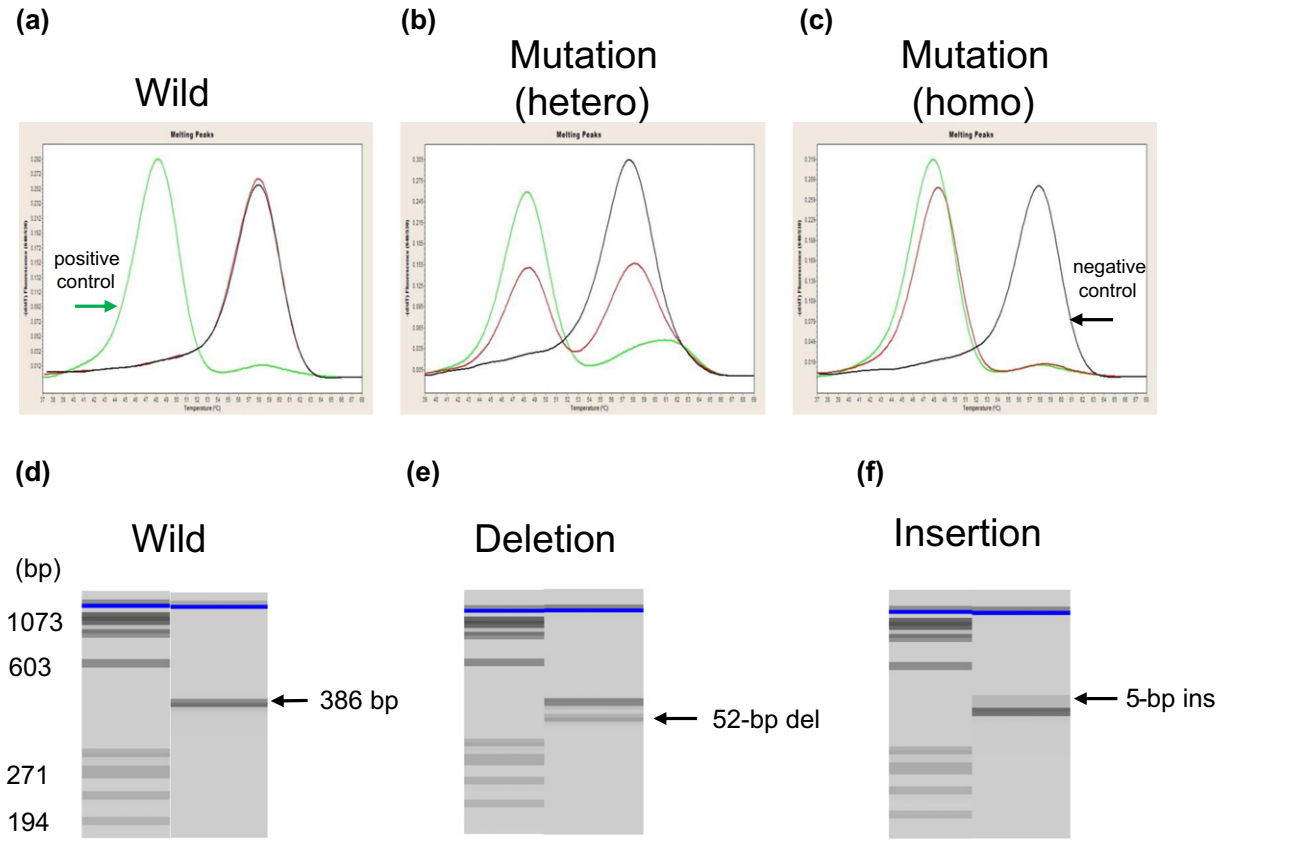

Fig. 1. Mutation analysis of JAK2 by melting curve analysis and the CALR gene by microchip electrophoresis. (a–c) Representative results of melting curve analysis for JAK2. Green line: positive control. Black line: negative control. Red line: representative samples. (a) wild-type JAK2 (b) heterozygous JAK2 mutation (c) homozygous JAK2 mutation. (d–f) Representative results of PCR for the CALR gene. (d) A single 386-bp band (arrow) was observed in specimens with the wild-type CALR gene. (e) A band smaller than that seen with the wild type was observed in the 52-bp deletion type. (f) An additional band was detected in the 5-bp insertion type. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

mutually exclusive of the presence of JAK2 or MPL mutations. Accordingly, the authors examined 211 additional JAK2/MPL-non-mutated cases and found that the CALR mutational frequency in JAK2/MPL-negative disease was 67% in ET and 88% in PMF. In consideration of the promising results presented in recent reports, diagnostic criteria used for MPN may be revised in the near future. In the present study, we examined the frequency and clinical correlations of the JAK2 and CALR mutations in MPN patients treated at our institution, and introduce our novel screening testing method utilizing high resolution melting (HRM) for use in clinical practice.

Table 1
Frequencies of JAK2 and CALR gene mutations in 177 cases.

		JAK2 mutation		CALR mutation	
Clinical diagnosis	No. of samples	Occurrence	Frequency (%)	Occurrence	Frequency (%)
MPN	99				
ET	43	24	56	14	43
PV	38	32	84	0	0
PMF	7	4	57	0	0
CML	11	0	0	0	0
Secondary or stress polycythemia	15	0	0	0	0
Reactive thrombocytosis	8	0	0	0	0
MDS	5	0	0	0	0
AML	3	0	0	0	0
Others	47	0	0	0	0

2. Materials and methods

2.1. Sample preparation

We examined samples from 177 patients previously investigated for the presence of the JAK2 mutation for differential diagnosis of MPN in patients treated at Nagasaki University Hospital, between 2008 and 2014. The final clinical diagnosis was PV in 38 cases, ET in 43, PMF in 7, secondary or stress polycythemia in 15, reactive thrombocytosis in 8, CML in 11, MDS in 5, AML in 3, and others in 47. Diagnosis of PMN or other diseases was performed at the time of the first observation in accordance with the WHO criteria presented in 2008.

This retrospective study received approval from the Ethics Committee of Nagasaki University Hospital (No. 16042540), and all procedures used were in accordance with the Helsinki Declaration, revised in 2000.

2.2. Detection of JAK2 V617F mutation using melting curve analysis

DNA was extracted from bone marrow samples or peripheral blood using QuickGene-800 (FUJIFILM, Japan). Codon 12 of the JAK2 gene was amplified using the LightCycler platform (Roche, Basel, Switzerland). Real-time PCR was performed using each specimen with 5 μL of purified DNA extract in a total reaction volume of 20 μL that included 2 μL of FastStart DNA Master Hybri Probe 10× reaction master mix (Roche) under the following conditions: forward primer, 5′-AAGCAGCAAGT ATGATGAGCAA-3′ (final concentration 0.5 μM); reverse primer 5′-AGCTGTGATCCTGAAACTGAA-3′ (final concentration 0.5 μM); FITC probe 5-GTAGTTTTACTTACTCTCGTCTC-FITC-3 (final concentration