Carlituculin and JAK2 Exon 12 Mutation Screening in Patients with Myeloproliferative Neoplasm's in Jeddah Region, Saudi Arabia

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Abstract

Purpose

The *JAK2V617F* mutation's discovery has largely facilitated the comprehension of the myeloproliferative neoplasms (MPNs) pathogenesis. In recent times, calreticulin (*CALR*) mutations have been detected in patients with negative MPNs for *JAK2V617F*. The *CALR* gene mutations have been reported in patients with negative *JAK2V617F*, primary myelofibrosis (PMF), and essential thrombocythemia (ET). This study analyzed the impact of *JAK2* Exon 12 and *CALR* common mutations on MPN patients from the Jeddah region.

Methods

An allele-specific polymerase chain reaction (PCR) method was used to screen four common mutations on Exon 12. Consequently, the results showed that none of the patients were positive for the Exon 12 mutation. Direct sequencing and PCR analysis were utilized to screen all patients for *CALR*.

Results

The impact of *CALR* and *JAK2* Exon 12 in 65 patients with a variety of MPN symptoms was investigated. This study included patients who had negative results, when previously screened for *JAK2V617F*. *CALR* mutations were identified in eight patients out of 65 *JAK2V617F* and *JAK2* Exon 12 negative patients in this study's cohort.

Conclusions

This is the first Saudi Arabian research that focused on screening *CALR* hotspot mutations, and this mutation exists. This fact highlights the importance of implementing diagnostic screening of *CALR* on MPN patients, in general, and patients with high platelet count, in particular. In addition, further screening of other predisposing genetic markers might facilitate the identification of an important genetic variant, which could aid in the understanding of disease pathogenesis.

Introduction

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of hematopoietic stem cell (HSC) and progenitor cell associated disorders, which involve chronic myeloid neoplasms and have the potential of disease progression to acute leukemia. An initial hit in HSC in the context of MPNs results in independence from normal cytokine regulation or hypersensitivity. In turn, this causes the excessive mature cell production with one or more blood cell lineages [1]. The new WHO classification of lymphoid and hematopoietic tissue-specific tumors specifies that BCR-ABL1-negative MPNs comprise a family of three hematological malignancies, which include essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF) [1, 2]. The discovery of a frequent point mutation in Exon 14 (V617F) of Janus kinase 2 (*JAK2*) [3, 4], which occurs in 95 percent of cases of PV and 60 percent of

cases of PMF and ET significantly advanced the characterization of classic MPNs' genetic background in 2005 [3–8]. The unique *JAK2V617F* mutation plays an important role in MPNs' pathogenesis, thereby resulting in the constitutive activation of JAK/STAT signaling and the subsequent stimulation of differentiation and proliferation of the myeloid lineages [9]. Furthermore, evidences prove that *JAK2* exon 12 mutations are present in approximately 5 percent of PV, who were negative for *JAK2* (V617F) [10, 11]. Moreover, between 5 and 10 percent of *JAK2* mutation (*JAK2*mut)-negative PMF and ET patients have point mutation on exon 10 of the thrombopoietin receptor gene (MPL) [12–16]. However, approximately 30 to 45 percent of BCR-ABL negative MPNs patients do not have MPL and JAK2 V617F mutations [17].

In 2013, Klampfl *et al.* and Nangalia *et al.* described how somatic mutations recurrently and exclusively affect the exon 9 of the calreticulin (*CALR*) gene [18, 19]. *CALR* mutations were mutually exclusive in the MPL and *JAK2*-non-mutated patients' subset [14]. This, in turn, confirms an MPN diagnosis. The impact of *CALR* in prognosis and clinical presentation continues to be partially comprehensible. Although several additional studies have described the presence of low frequencies of *CALR* mutations in different MPN-related diseases, they have not specified this presence with respect to other hematologic diseases.

The aim of this study is to investigate the prevalence of *CALR* and *JAK 2* Exon 12 mutations in *JAK2* non-mutated patients with suspected MPN from Jeddah City. This patient group included those who were previously screened for *JAK2V617F* in the CEGMR molecular genetic laboratory.

Methods

Patient Samples

This study includes 65 bone marrow or blood samples collected from patients diagnosed with MPNs at King Abdulaziz University Hospital. The samples were referred to Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Saudi Arabia for the molecular screening of *JAK2V617F*. The patient group in this study comprises 65 patients, which comprises other non MPN with thrombosis 25 (38.46 percent), 17 ET cases (26.15 percent), 4 PMF cases (6.15 percent), 9 PV cases (13.85 percent), 9 unclassified MPNs (13.85 percent), and 1 CMML (1.53 percent) who were diagnosed after the 2008 WHO classification of myeloid neoplasms. This study involved diligently documenting the patients' information, which comprises their hematological and diagnostic findings, that is, WBC, platelet, and RBC count as well as hemoglobin levels. Approval for this work was sought from CEGMR Bioethical committee with ethical no. HA-02-J-003.

Mutation Detection using Sanger Sequencing for CALR **and** JAK2 **exon14 (V617F).**

Genomic DNA was isolated from PB and BM using QIAmp DNA Blood mini kit (Qiagen, Germany). Subsequently, the concentration was quantified by Nanodrops 2000 spectrophotometer (Thermo Scientific, USA). *JAK2V617* and *CALR* Exon 8 and 9 were independently polymerase chain reaction (PCR) amplified independently by employing the following primer: *CALR* forward (5'-ACAACTTCCTCATCACCAACG-3'), reverse (5'-GGCCTCAGTCCAGCCCTG-3'), Exon 14 forward *JAK2*

Exon14 forward (5'-TTCTTTGAAGCAGCAAGTATGATGA-3'), and *JAK2* Exon14 reverse (5'-CTGACACCTAGCTGTGATCC-3').

The PCR was performed in 20µl volume, which comprised 1 µl of dNTPs, 2 µl of 10xbuffer, 1 µl forward primer and reveres primer each, 13.8 µl of nuclease water, 0.2 µl hot start DNA polymerase (5u/µl), and 1 µl (100 to 300 ng) of DNA template. The cycle protocol used in the PCR was as follows: initial denaturation at 95°C for 15 minutes followed by denaturation at 95°C for 30 seconds. Subsequently, the mixture was annealed with different temperatures for each gene for 40 cycles and this was followed by extension at 72°C for 10 minutes. After amplification, the product quality and size were visualized on 2 percent agarose gel, which was stained with ethidium bromide under ultraviolet (UV) light by utilizing the gel documentation system. The PCR product bands' molecular size were determined by extrapolating the 50 base pair (bp) scale DNA ladder loaded alongside to the bands. The aforementioned primers, an ABI 3730 XL automatic sequencer (Applied Biosystems), and the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) were used to directly sequence the PCR products.

Allele-specific PCR (AS-PCR) of JAK2 **Exon 12**

Multiplex PCR reactions, was used to amplify exon 12 using the following primes : **E12F**: 5'-CTCCTCTTTGGAGCAATTCA-3', **E12R**: 5'-GAGAACTTGGGAGTTGCGATA-3', **K539L**: 5'-CATATGAACCAAATGGTGTTTTCACTT-3', **N542-E543del**: 5'-CAAATGGTGTTTCACAAAATCAGAGATT-3', **F537-K539delinsL**: 5'-CATATGAACCAAATGGTGTTAATC-3', **H538QK539L**: 5'-CATATGAACCAAATGGTGTTTTCAATT-3'.

The reaction was performed using genomic DNA template 0.5 µl, and following the amplification reaction as illustrated in Table 1. The PCR procedure amplifies the exon 12 using primer set following the described protocol in Scott research [11].

Table 1
Master Mix Components for JAK2 Exon 12 Multiplex PCR.

Reagent	Set1		Set2		Set3	
	1 test	X 10	1 test	X 10	1 test	X 10
Injection water	18.8	188	19.3	193	19.3	193
10X PCR Buffer	2.5	25	2.5	25	2.5	25
10Mm dNTPs	0.5	5	0.5	5	0.5	5
Forward Primer E- 12	o.5	5	o.5	5	0.5	5
Reverse Primer E- 12	o.5	5	o.5	5	0.5	5
K539	0.5	5	-	-	-	-
H538QK539 L	o.5	5	-	-	-	-
N542-E543del	-	-	0.5	5	-	-
F537-K539 delins L	-	-	-	-	0.5	5
Taq polymerase	0.2	2	0.2		0.2	2
Sub total	24 µl	-	24 µl	-	24 µl	-
DNA	0.5	-	o.5	-	o.5	-
TOTAL volume	24.5 µl		24.5 µl		24.5 µl	

Results

Clinical Characteristic of Patients

This study was conducted by including 65 MPN patients, which comprises other non MPN with thrombosis 25 (38.46 percent), 17 ET cases (26.15 percent), 4 PMF cases (6.15 percent), 9 PV cases (13.85 percent), 9 unclassified MPNs (13.85 percent), and 1 CMML (1.53 percent) (Fig. 1). The patients' median age at diagnosis was 60 years. Furthermore, the patients were categorized and diagnosed using the WHO 2008 criteria [15].

The presence molecular evidence of BCR/ABL fusion transcripts and/or translocation t(9;22) (Philadelphia chromosome) as well as *JAK2V617F* were analyzed in the Center of Excellence in Genomic Medicine Research for all cases, and all the results were negative. Sequence analysis of *CALR* and *JAK2* Exon 12 was conducted for all 65 cases.

Mutation Screening of JAK2 Exon 12 by Allele-specific PCR

JAK2 exon 12 mutations (N542-E543, K539L, H538QK539L, and K539delinsl) were investigated in all 65 MPN patients' cohort. The result showed that non-exon 12 mutations were detected in all MPN patients who were investigated (Fig. 1).

CALR Exon 9 Screening

CALR frameshift mutation types 1 and 2 were investigated in all patients' cohort. The sequencing results detected two in-frame deletion mutations within *CALR* exon 9 in 12.3 percent of patients (eight out of 65) who are *JAK2V617F* and Exon 12 negative, which accounted for 29.4 percent (5 out of 17) of ET patients and 8 percent (2 out of 25) of non-MPN with thrombocytosis patients, and in 1 CMML patient (Fig. 2). Amongst the *CALR* frameshift mutations, four patients had typical type 1 mutations (L367fs*46) and a 52-bp deletion, while four patients had type 2 mutations (K385fs*47) 5 bp insertion. TYPE-1 mutation (L367fs*46) was detected in two out of five ET (11.5 percent) and 1 non-MPN with thrombocytosis patients. On the other hand, TYPE-2 mutation (K385fs*47) was detected in three out five ET patients, 1 CMML patient, and 1 non-MPN with thrombocytosis patient.

CALR Mutation with Clinical Characteristics

The patients with *CALR* mutations were Saudi adults who had been diagnosed with thrombocytosis or MPN and their molecular screening results for *JAK2V617F* and BCR/ABL were negative. Patients suffering from ET and patients with *CALR* frameshift mutations tend to have a higher platelet counts with normal hemoglobin levels. Moreover, the diseases of patients with ET who had *CALR* mutations did not progress to accelerated or blast phase disease in comparison with other MPNs. No differences in mutation rates were detected amongst men and women and there were no age-restricted differences.

Discussion

In the context of the classification of MPNs and MPN/myelodysplastic syndrome with thrombocytosis and ring sideroblasts, V617F, *JAK2*, *CALR* and Exon 12 mutations are critical biomarkers. At present, general guidelines for *JAK2* and *CALR* molecular testing in MPNs are unavailable. Several suggested indicators for these mutations' molecular testing in screening and diagnosis involve the following work-up: (1) clinically suspected PV, PMF, ET, and MDS/MPN with thrombocytosis and ring sideroblasts, (2) unexplained leukocytosis, and (3) unexplained splanchnic vein thrombosis [20].

Mutational testing does not only play an important role in the diagnosis and prognosis of the diseases but it also provides significant prognostic and other critical information. For instance, type 1 *CALR* mutations in PMF have been associated with superior survival rates than type 2 *CALR* and *JAK2* mutations [21]. Across all MPNs, *JAK2* mutations are related with higher hemoglobin, older age, leukocytosis, increased thrombotic events, and lower platelets [21, 22]. The *JAK2V617F*, Exon 12 and

CALR mutations are assessed by various molecular methods. This includes allele-specific (assessing for hotspot mutations) and sequencing-based methods.

We employed Sanger sequencing to detect *JAK2V617F* and *CALR* in this study, and allele-specific PCR was utilized to detect known mutations at Exon 12.

No mutations were detected amongst the 65 patients who were screened for exon 12 mutations. Although exon 12 mutations' prevalence is uncommon, the method used for detection of mutations might not have the sensitivity margin to detect exon 12 mutations.

Allele-specific tests, which assess hotspot mutations, might fail to detect other relevant variants that are less common, for example, *JAK2* Exon 12 mutations, and those that are found in approximately 3 percent of PVs, thereby giving false negatives. In addition, some allele-specific methods might be affected by near variant interferences, wherein the hotspot mutation's detection might be hindered by the interference of a second nearby single nucleotide variant.

The sensitivity of some techniques is less sensitive than that of others. Moreover, some MPNs (for example, ET) can present with low mutant allele fractions. Newer methods are more sensitive than Sanger sequencing, as it has the typical detection limit of 20 percent mutant allele fraction [23]. No universally accepted cutoffs are available for a positive result, even though some recommend the analytical sensitivity range between 1 and 3 percent mutant allele fraction for molecular assays [24, 25].

Mutation advent in the *CALR* gene changed the MPN landscape. Klampfl *et al.* in 2013 first recognized it as a somatic mutation in those patients who had MPNs but with no mutations in either MPL or *JAK2* [19]. *CALR* is a protein found in cytoplasm, endoplasmic reticulum, or cell surface. It maintains calcium hemostasis and regulates cell proliferation, apoptosis, and phagocytosis while facilitate accurate glycoprotein folding [26].

CALR mutation was identified in eight out of the total 65 patients who presented with thrombocytosis and had been suspected to have MPN. Amongst patients who had the *CALR* mutation, six patients were diagnosed as suffering from essential ET based on the 2016 WHO classification and diagnostic criteria for MPNs. Consequently, the *CALR* mutation types 1 and 2 were equally identified amongst those patients who were diagnosed as suffering from ET. The other two patients in which *CALR* mutations were detected were diagnosed as suffering from systemic lupus erythematous (SLE) and chronic myelomonocytic leukemia (CMML). No discernible difference could be detected in the disease's clinical phenotype amongst those with *CALR* and ET mutations.

Usually, the patients in whom heterozygous *CALR*-mutated PMF is detected are men whose age is comparatively younger than that of patients with *JAK2*-mutated cases. Furthermore, myeloproliferation in such cases is more specific to the megakaryocytic lineage and, therefore, presents with a more pronounced thrombocytosis. They usually have low white cell count and hemoglobin. Longer survival rates and low incidence of thrombotic complications are reported in this patient group. The prognostic

impact of *CALR* on PMF is limited to type 1 mutation while the prognosis in case of type 2 is similar to that of JAK2-mutated PMF [27].

The widespread impact of *CALR* mutation on MPNs, baseline characteristics, disease outcome, a patient's clinical behaviors, and benefits and risks in the long term warrants further exploration, as the impact of *CALR* mutation on MPNs is a recent scientific discovery. Prospective studies have to be conducted in order to outline the manner in which *CALR* mutation influences MPNs by following a detail-oriented approach toward the mutation's homozygous pattern. Several publications have analyzed and elucidated the *CALR* mutation screening methods, and it has even been suggested by some authors that fragment analysis determination might sufficiently fulfill the needs of routine diagnosis and aid in the development of real-time PCR detection methods [28]. Such screening methods do not facilitate precise characterization. Therefore, determining the accurate size of insertion or deletion might sometimes prove to be difficult through fragment analysis. This is an important issue, as this study has showcased that in-frame indel polymorphisms could be misinterpreted as mutations in case they are improperly characterized. Sanger sequencing was employed in this study, as mutation characterization is a critical factor in not only determining whether the alteration belongs to clinically relevant types, namely, 1 or 2, or to the type-1/2-like but also differentiating polymorphisms from point/nonsense mutations, which might play an important role in diagnosis.

Rare nonsense mutations, which indicate the loss of a variable number of negatively charged amino acids of the C-terminus, have been reported. This includes p.E380X, p.E374X, and p.K391X [29]. We consider both the simultaneous assessment of multiple mutations by next-generation sequencing and sequential testing algorithms as valid testing approaches. NGS facilitates the holistic assessment of targeted genes that are relevant to myeloid neoplasms. The genes that were included in this regard differ from one another based on the specific panel. Moreover, larger insertions and deletions (notably, type 1 mutations in *CALR* exons 9) might be missed based on the data analysis types used for the panel [30]. NGS' comprehensive nature will enable the reporting of less well-characterized variants of uncertain significance at other loci as well as pathogenic variants.

Our understanding of the molecular landscape of PV, PMF and ET is rapidly evolving. Recent studies have implicated other genes and pathways in disease progression and MPN pathogenesis [22]. Although many amongst such mutations are not specific to MPNs, they represent additional biomarkers that can be utilized to provide additional prognostic-specific information or prove clonality [22]. Molecular testing is simultaneously becoming increasingly complex—in terms of the associated technologies as well as the produced information. The facilitation of strong and effective communication between pathologists, clinicians, and molecular diagnosticians is important to properly integrate molecular data with clinical and pathologic findings.

Conclusions

This is the first Saudi Arabian research that focused on screening *CALR* hotspot mutations, and this mutation exists. This fact highlights the importance of implementing diagnostic screening of *CALR* on MPN patients, in general, and patients with high platelet count, in particular. In addition, further screening of other predisposing genetic markers might facilitate the identification of an important genetic variant, which could aid in the understanding of disease pathogenesis.

Declarations

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Author Contribution

SB participated in revising the clinical data and the disease diagnosis and drafted the manuscript. HAK, RF, RA and AE participated in analysis of data, helped in designing images, tables, critical review and drafted the manuscript. HA, ZA, HAS and EY carried out the experiment including DNA extraction, sequencing studies. AM performed data collection. HAK participated in designing the study, provided required reagents for the experiment and helped in drafting the manuscript. AA helped in providing the reagents, kits and other logistics in order to perform the study. All authors read and approved the final manuscript.

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conflict of Interest The authors have no competing interests.

Data availability The data supporting fndings of this study are available on request from the corresponding author. The data are not publicy available due to privacy or ethical restrictions.

Ethical approval Protocols for experiment on human samples were approved by ethical no. HA-02-J-003 in the Bioethical committee of CEGMR.

References

1. Vainchenker W, Delhommeau F, Constantinescu SN, Bernard OA (2011) New mutations and pathogenesis of myeloproliferative neoplasms. Blood 118(7):1723–1735
2. Sabattini E, Bacci F, Sagramoso C, Pileri SA (2010) WHO classification of tumours of haematopoietic and lymphoid tissues in 2008: an overview. Pathologica 102(3):83–87

3. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C et al (2005) A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 434(7037):1144–1148
4. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR et al (2005) A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 352(17):1779–1790
5. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S et al (2005) Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 365(9464):1054–1061
6. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ et al (2005) Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 7(4):387–397
7. Cross NC (2011) Genetic and epigenetic complexity in myeloproliferative neoplasms. Hematology Am Soc Hematol Educ Program 2011:208–214
8. Kvasnicka HM (2013) WHO classification of myeloproliferative neoplasms (MPN): A critical update. Curr Hematol Malig Rep 8(4):333–341
9. Levine RL, Pardanani A, Tefferi A, Gilliland DG (2007) Role of JAK2 in the pathogenesis and therapy of myeloproliferative disorders. Nat Rev Cancer 7(9):673–683
10. Lakey MA, Pardanani A, Hoyer JD, Nguyen PL, Lasho TL, Tefferi A et al (2010) Bone marrow morphologic features in polycythemia vera with JAK2 exon 12 mutations. Am J Clin Pathol 133(6):942–948
11. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR et al (2007) JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med 356(5):459–468
12. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M et al (2006) MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood 108(10):3472–3476
13. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M et al (2006) MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med 3(7):e270
14. Ahmed RZ, Rashid M, Ahmed N, Nadeem M, Shamsi TS (2016) Coexisting JAK2V617F and CALR Exon 9 Mutations in Myeloproliferative Neoplasms - Do They Designate a New Subtype? Asian Pac J Cancer Prev 17(3):923–926
15. Kilpivaara O, Levine RL (2008) JAK2 and MPL mutations in myeloproliferative neoplasms: discovery and science. Leukemia 22(10):1813–1817
16. Kralovics R (2008) Genetic complexity of myeloproliferative neoplasms. Leukemia 22(10):1841–1848
17. Grinsztejn E, Percy MJ, McClenaghan D, Quintana M, Cuthbert RJ, McMullin MF (2016) The prevalence of CALR mutations in a cohort of patients with myeloproliferative neoplasms. Int J Lab Hematol 38(1):102–106

18. Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC et al (2013) Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med 369(25):2391–2405
19. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD et al (2013) Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med 369(25):2379–2390
20. Boissinot M, Lippert E, Girodon F, Dobo I, Fouassier M, Masliah C et al (2006) Latent myeloproliferative disorder revealed by the JAK2-V617F mutation and endogenous megakaryocytic colonies in patients with splanchnic vein thrombosis. Blood 108(9):3223–3224
21. Tefferi A, Guglielmelli P, Larson DR, Finke C, Wassie EA, Pieri L et al (2014) Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. Blood 124(16):2507–2513; quiz 615
22. Tefferi A, Pardanani A (2015) Myeloproliferative Neoplasms: A Contemporary Review. JAMA Oncology 1(1):97–105
23. Wang YL, Vandris K, Jones A, Cross NC, Christos P, Adriano F et al (2008) JAK2 Mutations are present in all cases of polycythemia vera. Leukemia 22(6):1289
24. Bench AJ, White HE, Foroni L, Godfrey AL, Gerrard G, Akiki S et al (2013) Molecular diagnosis of the myeloproliferative neoplasms: UK guidelines for the detection of JAK2 V617F and other relevant mutations. Br J Haematol 160(1):25–34
25. Sidon P, El Housni H, Dessars B, Heimann P (2006) The JAK2V617F mutation is detectable at very low level in peripheral blood of healthy donors. Leukemia 20(9):1622
26. Luo W, Yu Z (2015) Calreticulin (CALR) mutation in myeloproliferative neoplasms (MPNs). Stem Cell Investig 2:16
27. Guglielmelli P, Nangalia J, Green AR, Vannucchi AM (2014) CALR mutations in myeloproliferative neoplasms: hidden behind the reticulum. Am J Hematol 89(5):453–456
28. Jones AV, Ward D, Lyon M, Leung W, Callaway A, Chase A et al (2015) Evaluation of methods to detect CALR mutations in myeloproliferative neoplasms. Leuk Res 39(1):82–87
29. Lim KH, Chang YC, Gon-Shen Chen C, Lin HC, Wang WT, Chiang YH et al (2015) Frequent CALR exon 9 alterations in JAK2 V617F-mutated essential thrombocythemia detected by high-resolution melting analysis. Blood Cancer J 5:e295
30. Kluk MJ, Lindsley RC, Aster JC, Lindeman NI, Szeto D, Hall D et al (2016) Validation and Implementation of a Custom Next-Generation Sequencing Clinical Assay for Hematologic Malignancies. J Mol Diagn 18(4):507–515

Figures

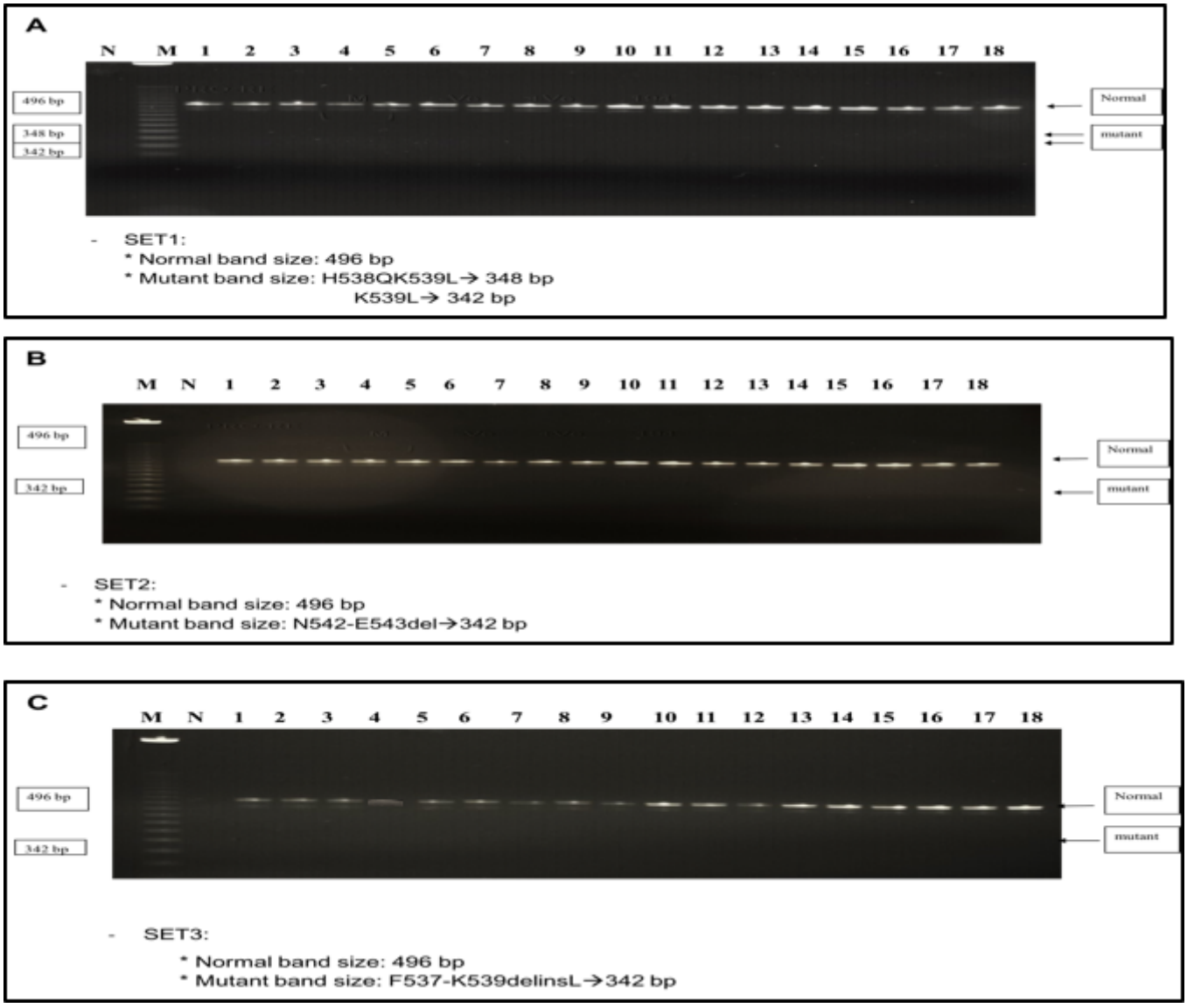

Figure 1

PCR amplification of exon 12 DNA using multiplex PCR. (A) Represents Set 1 [the specific primers were K539L (342 bp) and H538QK539L (348 bp)]. (B) Set 2 [the specific primer was N542-E543 (342 bp)]. (C) Set 3 [the specific primer was K539delinsl (342 bp)]. N: Negative control; M: DNA marker (50 pb), Lanes (1–18): Amplified DNA from 18 samples.

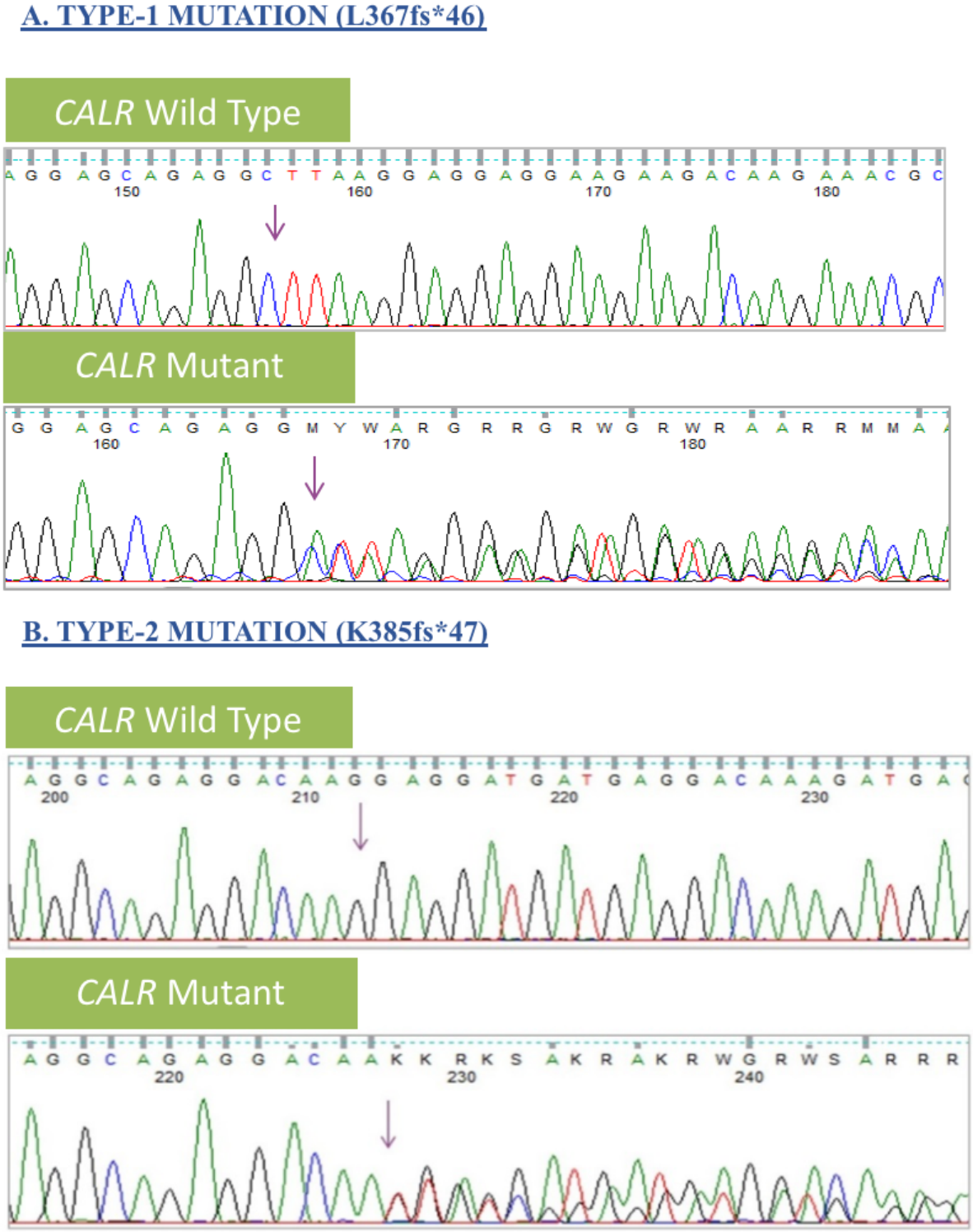

Figure 2

Sanger sequencing for CALR exon 9 mutation. (A) Normal control vs Positive samples with in frame deletion mutation (L367fs*46), (B) Normal control vs Positive sample with in frame deletion mutation (K385fs*47).