Clinical and Hematological Relevance of JAK2V617F, CALR, and MPL Mutations in Vietnamese Patients with Essential Thrombocythemia

Hoang Anh Vu1, Tran Thi Thao2, Cao Van Dong3, Nguyen Lam Vuong4, Ho Quoc Chuong1, Phan Nguyen Thanh Van3, Huynh Nghia2,3, Nguyen Tan Binh3, Phu Chi Dung3, Phan Thi Xinh2,3,*

Abstract

Background: The picture of Vietnamese patients with essential thrombocythemia (ET) remains mostly undetermined. Our study intended to determine the frequency of JAK2V617F, CALR exon 9, and MPL exon 10 mutations as well as to analyze clinical characteristics associated with different mutational status in Vietnamese ET patients. Methods: We explored mutations of JAK2V617F, MPL, and CALR from 395 patients using allele specific oligonucleotide – polymerase chain reaction and Sanger sequencing techniques; then, the clinical and hematological features were compared according to mutation patterns. Results: We found that JAK2V617F, CALR exon 9, and MPL exon 10 mutations were present in 56.2%, 27.6%, and 1% of the 395 patients with ET, respectively. Twelve different types of CALR mutation were detected in 109 patients, with the CALR type 1 mutation (c.1099_1150del; L367fs*46) was the most common, followed by CALR type 2 mutation (c.1154_1155insTTGTC; K385fs*47). The JAK2V617F-positive patients had older age, higher white blood cell counts and higher hemoglobin levels but lower platelet counts than patients with CALR mutations or patients negative for triple tests. There was no significant difference regarding sex ratio, white blood cell counts, platelet counts and hemoglobin levels among CALR mutation subtypes. Conclusion: we reported high frequency of JAK2V617F, CALR, and MPL mutations in Vietnamese patients with ET and underscored the importance of combined genetic tests for diagnosis and classification of ET into different subtypes.

Keywords: Essential thrombocythemia- JAK2V617F- CALR- MPL- Vietnam

Asian Pac J Cancer Prev, 20 (9), 2775-2780

Introduction

Essential thrombocythemia (ET), a subtype of the BCR-ABL1-negative myeloproliferative neoplasms (MPNs), is a clonal hematopoietic stem cell disorder characterized by an isolated thrombocytosis and associated with complications such as thrombosis, hemorrhage, and progression to myelofibrosis or acute myeloid leukemia. The three most common BCR-ABL1-negative MPNs are polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). In 2005, the discovery of JAK2V617F mutation created a breakthrough in the diagnosis of BCR-ABL1-negative MPNs (Campbell et al., 2005; James et al., 2005; Kralovics et al., 2005). The JAK2V617F was present in roughly 90% of patients with PV and in 50% to 60% of those with ET or PMF. In addition, MPL exon 10 mutations (mainly involving codon W515) were found in 5% to 10% of patients with JAK2V617F-negative ET and PMF (Pardanani et al., 2006; Pikman et al., 2006). Recently, novel frameshift mutations in exon 9 of the calreticulin (CALR) gene were identified in ET or PMF patients without JAK2 and MPL mutations (Nangalia et al., 2013). Approximately 70 different indels in CALR exon 9 were classified into CALR type 1 (c.1099_1150del; L367fs*46: 50% of all types), CALR type 2 (c.1154_1155insTTGTC; K385fs*47: 30% of all types), and CALR other types (Al Assaf et al., 2015; Kim et al., 2015). The somatic mutations in JAK2, CALR, and MPL were included in the World Health Organization (WHO) classification of MPNs (Arber et al., 2016). Several studies have shown that JAK2V617F-mutated ET patients had older age, lower platelet counts, higher hemoglobin levels, higher leukocyte counts, and higher thrombotic risk compared with CALR-mutated cases (Al Assaf et al., 2015; Cazzola and Kralovics, 2014; Tefferi et al., 2014). However, CALR-mutated ET had a relatively...
higher risk of myelofibrotic transformation, especially in cases with CALR type 1 mutation (Pietra et al., 2016).

To the best of our knowledge, the characteristics of Vietnamese patients with ET remains mostly undetermined. In this study, we investigated the profiles of JAK2V617F, MPL, and CALR mutations in Vietnamese ET patients using allele specific oligonucleotide – polymerase chain reaction (ASO-PCR) and conventional Sanger sequencing method. The clinical and hematological features were compared according to mutation patterns.

Materials and Methods

Patients and samples

This was a retrospective study of 395 patients diagnosed with ET between 2008 and 2017 at Blood Transfusion and Hematology Hospital at Ho Chi Minh City, Vietnam. The diagnosis of ET was established based on the 2008 WHO diagnostic criteria (Campo et al., 2011). In brief, patient was diagnosed with ET when he/she had thrombocytosis, megakaryocyte proliferation, and did not meet WHO criteria for other MPNs, myelodysplastic syndrome (MDS) or myeloid neoplasm. Clinical and hematological findings at diagnosis were obtained by reviewing the medical records. Written informed consents for mutation analyses were obtained from patients enrolled in this study. Genomic DNA was extracted from peripheral blood samples using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer’s instruction.

Mutation analysis

All primers used in this study were newly designed. All 395 samples were assessed for JAK2V617F status using ASO-PCR technique. Genomic DNA was amplified in a 35-cycle PCR reaction at an annealing temperature of 60°C using three primers. The reaction contained 25 – 50 ng of genomic DNA, 1X PCR Buffer, 200 µM each dNTP, 0.1 µM common forward primer, 0.1 µM each of reverse primers. The mutant allele showed two bands at 453 base pairs (bp) and 279 bp, while the wild-type allele had only one band at 453 bp. Primers were as follows: reverse wild-type – specific primer, 5'-atgtctccttttcacaagat-3'; reverse mutant – specific primer, 5'-gtttactctctctctctccacaaaa-3'; and common forward primer, 5'-ctctcagagaagttgagccagga-3'.

Patients with non-mutated JAK2V617F were further evaluated for CALR exon 9 and MPL exon 10 mutations using Sanger sequencing method. The CALR exon 9 was amplified with primers CALR-F (5’-gaaccccgctgcaaaagcagc-3’) and CALR-R (5’-agacccacacatatggccggcagg-3’); while MPL exon 10 was amplified with primers MPL-F (5’-ttgtctcctcacaacacggtgcc-3’) and MPL-R (5’-cacaagcagcaagcagaaagc-3’). Each reaction consists of 1X PCR Buffer, 1.5 mM MgCl2, 200 µM each dNTP, 0.5 U Taq Hot Start Polymerase (Takara Bio), 0.1 µM each forward and reverse primers, and 25 – 50 ng of genomic DNA. PCR involved an initial denaturation at 98°C for 3 min followed by 40 cycles of 98°C for 10 sec, 60°C for 30 sec, and 72°C for 1 min with a final elongation of 72°C for 5 min. PCR products were checked for size and purity using 1.5% agarose gel electrophoresis. PCR products were purified enzymatically using ExoSAP IT™ PCR Product Cleanup Reagent (Thermo Scientific) for removal of excess primers and dNTPs prior to Sanger sequencing using a BigDye Terminator v3.1 Kit and ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). PCR fragments were sequenced and analyzed in both directions.

Statistical analysis

The clinical and hematological findings were summarized by each of the four groups of mutational status (JAK2, CALR, MPL, and triple-negative) and were compared between each pair of these groups using two-sided Fisher’s exact test for categorical variables and Mann-Whitney U test for numeric variables, where appropriate. The thrombotic-event-free survival rate was described by mutational status using Kaplan-Meier estimate. Statistical significance was defined as P-value less than 0.05. All statistical analyses were performed using the statistical software R version 3.4.4.

Results

Baseline clinical characteristics and prevalence of mutation

Among 395 patients diagnosed with ET, the follow-up duration ranged from 1 to 13 years, with the median length of follow-up of 3 years. The baseline clinical characteristics are shown in Table 1. There were more females than males (249/146). The median age was 54 years and more than 75% of the patients were middle-aged or older. There were 34 patients (8.6%) with history of arterial thrombotic diseases. According to the IPSET-thrombosis risk score, 130 patients (32.9%) had high risk and 112 patients (28.4%) had intermediate risk of thrombosis. The laboratory data showed normal median values of red blood cell (RBC) counts, hemoglobin (HGB) concentration, and white blood cell (WBC) counts. The median platelet count was 1037 × 10^9/L. There were also high values of megakaryocytes, lactate dehydrogenase (LDH), and serum uric acid.

Two hundred twenty-two patients (56.2%), 109 patients (27.6%), and 4 patients (1%) harbored JAK2V617F mutation, CALR mutations, and MPL mutations, respectively; leaving 60 patients (15.2%) negative for all three mutational tests. Of 109 CALR-mutated patients, CALR type 1 mutation (c.1099_1150del) was the most common, accounting for 61 patients (56%). Thirty-six patients (33%) carried CALR type 2 mutation (c.1154_1155insTTGTC). In the remaining 12 cases (11%), ten types of CALR mutations were detected as shown in Table 2. Four different types of MPL exon 10 mutations detected were S505N, W515K, W515L, and W515S.

Clinical characteristics with different mutational status

Clinical characteristics by mutational groups are shown in Table 3. There was no significant difference in sex ratio between groups. Compared to JAK2-mutated
JAK2, CALR, MPL Mutations in Vietnam

patients (JAK2 group), CALR-mutated (CALR group) and triple-negative patients (triple-negative group) were significantly younger (JAK2 group: 57 years; CALR group: 51 years; and triple-negative group: 45 years), had lower risk of thrombotic events based on IPSET-thrombosis risk score, showed lower RBC counts (JAK2 group: 4.8 × 10¹²/L; CALR group: 4.2 × 10¹²/L; and triple-negative group: 4.2 × 10¹²/L), lower HGB levels (JAK2 group: 13.2 g/dL; CALR group: 11.8 g/dL; and triple-negative group: 11.5 g/dL), lower WBC counts (JAK2 group: 14 × 10⁹/L; CALR group: 9.7 × 10⁹/L; and triple-negative group: 11.3 × 10⁹/L), higher platelet counts (JAK2 group: 950 × 10⁹/L; CALR group: 1207 × 10⁹/L; and triple-negative group: 1153 × 10⁹/L), and lower serum uric acid concentration (JAK2 group: 335 mg/dL; CALR group: 296 mg/dL; and triple-negative group: 302 mg/dL). No significant difference was observed concerning hepatomegaly, splenomegaly, and thrombotic events between these three groups. Two patients died

Table 1. Baseline Characteristics and Prevalence of Mutations in Patients with Essential Thrombocythemia

| Characteristics          | Summary statistics |
|--------------------------|--------------------|
| Number of patients, n    | 395                |
| Male, n (%)              | 146 (37.0)         |
| Age (years), median (IQR)| 54 (41, 66)        |
| Comorbidities, n (%)     |                    |
| - Hypertension           | 85 (21.5)          |
| - Dislipidemia           | 38 (9.6)           |
| - Arterial thrombosis    | 34 (8.6)           |
| - Diabetes               | 19 (4.8)           |
| IPSET-thrombosis risk, n (%)|            |
| - Low                    | 153 (38.7)         |
| - Intermediate           | 112 (28.4)         |
| - High                   | 130 (32.9)         |
| Laboratory data, median (IQR) |                |
| - RBC, × 10¹²/L          | 4.5 (4.0, 4.9)     |
| - HGB, g/dL              | 12.5 (11.2, 13.9)  |
| - WBC, × 10⁹/L           | 12.1 (9.6, 16.4)   |
| - Platelets, × 10⁹/L     | 1037 (793, 1342)   |
| - Megakaryocyte¹         | 80 (50, 100)       |
| - LDH, IU/L²             | 258 (217, 363)     |
| - Acid uric, mg/dL³      | 318 (260, 385)     |
| Mutation profiles, n (%) |                    |
| - JAK2                   | 222 (56.2)         |
| - CALR                   | 109 (27.6)         |
| CALR type 1              | 61 (15.4)          |
| CALR type 2              | 36 (9.1)           |
| CALR other types         | 12 (3.0)           |
| - MPL                    | 4 (1.0)            |
| - Triple-negative        | 60 (15.2)          |

¹, The number of patients was 315; ², The number of patients was 362; ³, HGB, hemoglobin; IPSET, International Prognostic Score for Thrombosis in Essential Thrombocythemia; IQR, interquartile range; LDH, lactate dehydrogenase; RBC, red blood cell; WBC, white blood cell.

Table 2. Mutational Characteristics of Patients with CALR Mutation

| CALR mutation type | n   | % |
|--------------------|-----|---|
| c.1099_1150del     | 61  | 56|
| c.1154_1155insTTGTC| 36  | 33|
| c.1100_1145del     | 3   | 11|
| c.1105_1138del     | 1   |   |
| c.1121_1139del     | 1   |   |
| c.1124_1142del     | 1   |   |
| c.1147_1151TGTT    | 1   |   |
| c.1149_1150insCAGAG| 1   |   |
| c.1149_1154>TCCTTGTC| 1 | 1|
| c.1153delA         | 1   |   |
| c.1116_1146del     | 1   |   |
| c.1129_1140>CCTTGCGA| 1 | 1|
| Total              | 109 | 100 |

Figure 1. Kaplan-Meier Curves for Thrombotic-event-free Survival by Mutational Status

Asian Pacific Journal of Cancer Prevention, Vol 20 2777
Table 3. Clinical and Laboratory Features Stratified by Mutational Status

| Characteristics       | JAK2 (1) | CALR (1) | MPL (1) | Triple-negative (2) | p1 vs 2 | p1 vs 3 | p1 vs 4 | p2 vs 3 | p2 vs 4 | p3 vs 4 |
|-----------------------|----------|----------|---------|---------------------|---------|---------|---------|---------|---------|---------|
| Number of patients, n | 222      | 109      | 4       | 60                  |         |         |         |         |         |         |
| Male, n (%)           | 87 (39.2)| 35 (32.1)| 3 (75.0)| 21 (35.0)           | 0.227   | 0.304   | 0.654   | 0.110   | 0.735   | 0.144   |
| Age, years            | 57 (45.68)| 51 (41.61)| 71 (56.77)| 45 (32.59)           | 0.005   | 0.379   | <0.001  | 0.186   | 0.065   | 0.138   |
| IPSET-thrombosis risk group, n (%) |         |         |         |         | <0.001 | <0.001 | 0.096   | 0.630   | 0.056   |         |
| - Low                 | 1 (0.5)  | 95 (87.2)| 2 (50.0)| 55 (91.7)           |         |         |         |         |         |         |
| - Intermediate        | 98 (44.1)| 10 (9.2) | 1 (25.0)| 3 (5.0)             |         |         |         |         |         |         |
| - High                | 123 (55.4)| 4 (3.7)  | 1 (25.0)| 2 (3.3)             |         |         |         |         |         |         |
| RBC, \(10^{12}/L\)    | 4.8 (4.3, 5.3)| 4.2 (3.9, 4.5)| 4.4 (3.8, 4.9)| 4.2 (3.7, 4.5) | <0.001 | 0.282   | <0.001  | 0.756   | 0.507   | 0.657   |
| HGB, g/dL             | 11.2 (11.8, 14.4)| 11.8 (10.8, 12.8)| 11.2 (10.2, 12.4)| 11.5 (10.0, 12.6) | <0.001 | 0.101   | <0.001  | 0.603   | 0.112   | 0.989   |
| WBC, \(10^{9}/L\)     | 9.7 (8.1, 11.9)| 6.3 (5.3, 7.5)| 11.3 (9.6, 13.8)| <0.001 | 0.001   | <0.001  | 0.014   | 0.017   | 0.007   |
| Platelets, \(10^{11}/L\) | 1207 (900, 1477)| 1111 (938, 1318)| 1153 (998, 1453)| <0.001 | 0.291   | <0.001  | 0.852   | 0.948   | 0.760   |
| Megakaryocyte         | 60 (30, 100)| 60 (30, 100)| 30 (30, 30)| 80 (58, 100)        | 0.015   | 0.200   | 0.391   | 0.370   | 0.011   | 0.162   |
| LDH, IU/L             | 263 (223, 352)| 230 (197, 434)| 249 (190, 398)| 0.796 | 0.679   | 0.250   | 0.579   | 0.189   | 0.856   |
| Acid uric, mg/dL      | 296 (242, 352)| 341 (294, 373)| 302 (242, 364)| <0.001 | 0.804   | 0.030   | 0.510   | 0.689   | 0.721   |
| Hepatomegaly, n (%)   | 10 (4.5) | 4 (3.7)  | 3 (5.0) | 1 1 1 0.700 |         |         |         |         |         |         |
| Splenomegaly, n (%)   | 29 (13.1)| 7 (6.4)  | 0 (0)   | 4 (6.7)             | 0.090   | 1       | 0.256   | 1       | 1       | 1       |
| Thrombotic events, n (%) | 6 (2.7) | 2 (1.8) | 2 (50.0) | 0 (0) | 1 0.006 | 0.348   | 0.006   | 0.539   | 0.003   |
| Mortality, n (%)      | 1 (0.5)  | 1 (0.9)  | 0 (0)   | 0 (0)               | 0.551   | 1       | 1       | 1       | 1       | -       |

Summary statistic is absolute count (%) for categorical variables and median (IQR) for continuous data; P values were calculated between patients in each pair of mutations. Significance values are in boldface; HGB, hemoglobin; IPSET, International Prognostic Score for Thrombosis in Essential Thrombocythemia; IQR, interquartile range; LDH, lactate dehydrogenase; RBC, red blood cell; WBC, white blood cell.

during follow-up period. Kaplan-Meier estimates for thrombotic-event-free survival by mutational status were shown in Figure 1. Among four cases with MPL mutation, two experienced thrombotic events with no mortality. There was no significant difference among JAK2, CALR, and triple-negative groups regarding the event-free survival estimate.
Clinical characteristics with different CALR mutation subtypes

The clinical characteristics by CALR mutation subtypes are shown in Table 4. While patients with CALR types 1 and types 2 were younger than other CALR types, there was no significant difference concerning sex ratio and IPSET-thrombosis risk score between these subtypes. Compared with CALR type 1, CALR type 2 patients seemed to have lower WBC counts, higher platelet counts, and lower LDH concentration; however, the differences were not statistically significant. Hepatomegaly, splenomegaly, thrombotic events, and mortality were rare in all subtypes.

Discussion

This is the first comprehensive study to describe the profile of Vietnamese patients with ET. Using ASO-PCR and conventional Sanger sequencing method, we found that 84.8% of 395 ET patients carried JAK2V617F, CALR, or MPL mutations, underscoring the importance of combined genetic tests for diagnosis of ET patients. The JAK2V617F was the most frequent mutation (56.2%), followed by CALR mutation (27.6%), which is consistent with Chinese (Lin et al., 2015), Japanese (Misawa et al., 2018), Argentinian (Ojeda et al., 2018), and American (Tefferi et al., 2014) ET populations. However, the frequency of CALR exon 9 mutations in our study is higher than that of Thai (Limsuwanachot et al., 2017), Korean (Kim et al., 2015), Italian (Rotunno et al., 2014), Polish (Wojtaszewska et al., 2015), and Brazilian (Nunes et al., 2015) ET patients; in those populations the mutation rate of CALR exon 9 mutations ranged from 12.5% to 15.5%. Twelve different types of CALR mutation including deletion, insertion and complex indels were found in our study. Type 1 (c.1099_1150del), type 2 (c.1154_1155insTTGTC), and other indels were detected in 56%, 33%, and 11%, respectively, in good agreement with previous reports (Al Assaf et al., 2015; Klampfl et al., 2013; Nangalia et al., 2013; Tefferi et al., 2014).

Among four different types of MPL exon 10 mutations detected (S505N, W515K, W515L and W515S), S505N was reported as a founder mutation in several pedigrees with familial thrombocytosis (Ding et al., 2004). However, it was also found as an acquired somatic mutation in rare ET patients (Beer et al., 2008; Vainchenker and Kralovic, 2017). In this study, the patient carrying MPLS505N was an 80-year-old man with HGB level of 10.2 g/dL, platelet count of 1,421 x 10^9/L, and WBC count of 5.71 x 10^9/L at diagnosis. He had history of thrombotic event and developed secondary bone marrow fibrosis three years after diagnosis.

Among JAK2V617F, CALR and triple-negative groups, we showed here that triple-negative ET patients were the youngest, quite similar to previous studies (Al Assaf et al., 2015; Ojeda et al., 2018). Also, consistent with previous reports, patients with JAK2 mutations had significantly higher HGB level, higher RBC and WBC counts, and higher thrombosis risk score, but lower platelet counts compared with patients with CALR mutations or triple-negative for mutations (Al Assaf et al., 2015; Ojeda et al., 2018; Rumi et al., 2014; Tefferi et al., 2014). When comparing the clinical and hematological findings among CALR type 1, CALR type 2, and other CALR types, we found that three CALR mutation groups were similar in their sex ratio, IPSET-thrombosis risk score, HGB level and WBC counts. In a similar cohort study of 402 ET patients, Tefferi et al concluded that patients with CALR type 2 had significantly higher platelet count compared with CALR type 1 (Tefferi et al., 2014). In our study, patients with CALR type 2 showed a tendency of higher platelet counts; however, there was no significant difference among these three CALR mutation groups (Table 3).

Within a 3-year median time of follow-up, thrombotic events and mortality were rare, with two death cases and ten thrombotic events. Long-term follow-up is required to further explore thrombotic events and mortality rate based on mutational groups. A limitation of our study is that this was a single-center retrospective study with a limited sample size. Therefore, only four patients harbored MPL mutations detected, with two of them experiencing thrombotic events. Although the prevalence of thrombotic events in the MPL mutation group was higher than in other groups, we could not conclude that this group was associated with worse outcome due to small number of patients.

In conclusion, this study is the first comprehensive investigation of gene mutations in Vietnamese patients with ET. The combined genetic tests can clarify approximately 85% of the ET patients with JAK2V617F, CALR exon 9, and MPL exon 10 mutations, which might improve the diagnosis and classification of ET in Vietnam.

Acknowledgements

The authors would like to thank Dr. Nguyen Thi Hong Hoa for assistance in providing samples and patient information.

Conflict of interest

The authors have no conflicts of interest to declare.

Funding information

No specific funding was available for this project.

Ethical issue

The study was approved by the ethics committee of the University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam (17551-DHYD).

References

Al Assaf C, Van Obbergh F, Billiet J, et al (2015). Analysis of phenotype and outcome in essential thrombocythemia with CALR or JAK2 mutations. Haematologica, 100, 893-7.
Arber DA, Orazi A, Hasserjian R, et al (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood, 127, 2391-405.
Beer PA, Campbell PJ, Scott LM, et al (2008). MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. Blood, 112, 141-9.
Campbell PJ, Scott LM, Buck G, et al (2005). Definition of subtypes of essential thrombocythaemia and relation to
polycythaemia vera based on JAK2 V617F mutation status: a prospective study. *Lancet*, **366**, 1945-53.

Campos E, Swerdlow SH, Harris NL, et al (2011). The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood*, **117**, 5019-32.

Cazzola M, Kralovics R (2014). From Janus kinase 2 to calreticulin: the clinically relevant genomic landscape of myeloproliferative neoplasms. *Blood*, **123**, 3714-9.

Ding J, Komatsu H, Wakita A, et al (2004). Familial essential thrombocytemia associated with a dominant-positive activating mutation of the c-MPL gene, which encodes for the receptor for thrombopoietin. *Blood*, **103**, 4198-200.

James C, Ugo V, Le Couédic JP, et al (2005). A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*, **434**, 1144-8.

Kim SY, Im K, Park SN, et al (2015). CALR, JAK2, and MPL mutation profiles in patients with four different subtypes of myeloproliferative neoplasms: primary myelofibrosis, essential thrombocytemia, polycythemia vera, and myeloproliferative neoplasm, unclassifiable. *Am J Clin Pathol*, **143**, 635-44.

Klampfl T, Gisslinger H, Harutyunyan AS, et al (2013). Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med*, **369**, 2379-90.

Kralovics R, Passamonti F, Buser AS, et al (2005). A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*, **352**, 1779-90.

Limsuwanachot N, Rerkamnuaychoke B, Chuncharunee S, et al (2017). Clinical and hematological relevance of JAK2 V617F and CALR mutations in BCR-ABL-negative ET patients. *Hematology*, **22**, 599-606.

Lin Y, Liu E, Sun Q, et al (2015). The prevalence of JAK2, MPL, and CALR mutations in Chinese patients with BCR-ABL1-negative myeloproliferative neoplasms. *Am J Clin Pathol*, **144**, 165-71.

Misawa K, Yasuda H, Araki M, et al (2018). Mutational subtypes of JAK2 and CALR correlate with different clinical features in Japanese patients with myeloproliferative neoplasms. *Int J Hematol*, **107**, 673-80.

Nangalia J, Massie CE, Baxter EJ, et al (2013). Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med*, **369**, 2391-405.

Nunes DP, Lima LT, Chauffaille Mde L, et al (2015). CALR mutations screening in wild type JAK2(V617F) and MPL(W515K/L) Brazilian myeloproliferative neoplasm patients. *Blood Cells Mol Dis*, **55**, 236-40.

Ojeda MJ, Bragos IM, Calvo KL, et al (2018). CALR, JAK2 and MPL mutation status in Argentinean patients with BCR-ABL1- negative myeloproliferative neoplasms. *Hematology*, **23**, 208-11.

Pardanani AD, Levine RL, Lasho T, et al (2006). MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood*, **108**, 3472-6.

Pietra D, Rumi E, Ferretti VV, et al (2016). Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. *Leukemia*, **30**, 431-8.

Pikman Y, Lee BH, Mercher T, et al (2006). MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med*, **3**, 270.

Rotunno G, Mannarelli C, Guglielmelli P, et al (2014). Impact of calreticulin mutations on clinical and hematological phenotype and outcome in essential thrombocytemia. *Blood*, **123**, 1552-5.

Rumi E, Pietra D, Ferretti V, et al (2014). JAK2 or CALR mutation status defines subtypes of essential thrombocytemia with substantially different clinical course and outcomes. *Blood*, **123**, 1544-51.