CALR, JAK2 and MPL mutation status in Argentinean patients with BCR-ABL1-negative myeloproliferative neoplasms

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ABSTRACT

Objectives: To establish the frequency of JAK2, MPL and CALR mutations in Argentinean patients with BCR-ABL1-negative myeloproliferative neoplasms (MPN) and to compare their clinical and haematological features.

Methods: Mutations of JAK2V617F, JAK2 exon 12, MPL W515L/K and CALR were analysed in 439 Argentinean patients with BCR-ABL1-negative MPN, including 176 polycythemia vera (PV), 214 essential thrombocythemia (ET) and 49 primary myelofibrosis (PMF).

Results: In 94.9% of PV, 85.5% ET and 85.2% PMF, we found mutations in JAK2, MPL or CALR. 74.9% carried JAK2V617F, 12.3% CALR mutations, 2.1% MPL mutations and 10.7% were triple negative. In ET, nine types of CALR mutations were identified, four of which were novel. PMF patients were limited to types 1 and 2, type 2 being more frequent.

Discussion: In ET, patients with CALR mutation were younger and had higher platelet counts than those with JAK2V617F and triple negative. In addition, JAK2V617F patients had high leucocyte and haemoglobin values compared with CALR-mutated and triple-negative patients. In PMF, patients with mutant CALR were associated with higher platelet counts.

Conclusion: Our study underscores the importance of JAK2, MPL and CALR genotyping for accurate diagnosis of patients with BCR-ABL1-negative MPN.

KEYWORDS

Myeloproliferative neoplasms; polycythemia vera; essential thrombocythemia; primary myelofibrosis; calreticulin; JAK2V617F; MPL

Introduction

The most frequent BCR-ABL1-negative myeloproliferative neoplasms (MPN) include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), according to the 2008 World Health Organization (WHO) classification criteria [1]. The understanding of the genetic basis of these disorders began in 2005, when the JAK2V617F mutation was described as the first recurrent molecular abnormality in BCR-ABL1-negative MPN [2–6]. This mutation is present in about 95% of patients with PV and in about 50–60% of those with ET or PMF. Mutations in JAK2 exon 12 and MPL exon 10 were subsequently reported in subsets of patients. JAK2 exon 12 mutations have been detected in most of the remaining cases with PV [7–10], while MPL exon 10 mutations (mainly involving codon W515) occur in 5–10% of patients with JAK2V617F-negative ET or PMF [11–14]. In 2013, somatic mutations in CALR gene, encoding calreticulin, were found in most patients with ET or PMF with JAK2 and MPL wild-type, in a mutually exclusive pattern with JAK2 and MPL mutations. Mutant CALR is the result of frameshift mutations caused by exon 9 deletions or insertions, type-1, 52-bp deletion (p.L367Fs*46), and type-2, 5-bp TTGTC insertion (p.K385fs*47) variants constitute more than 80% of these mutations [15–17].

The aim of the present study is to establish the frequency of major mutations in the JAK2, MPL and CALR genes in Argentinean patients with BCR-ABL1-negative MPN. The clinical and haematological features of ET and PMF patients were also compared according to the driver mutations displayed.

Materials and methods

This study was approved by the ethical committee at Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000, and samples were obtained after patients had provided written informed consent. From January 2008 to December 2016, bone marrow or peripheral blood samples from 439 BCR-ABL1-negative MPN patients were referred to our centre. According to the 2008 WHO criteria [2], 176 out of 439 subjects had PV, 214 had ET and 49 PMF. For each patient, a sample of genomic DNA was isolated and relevant laboratory information at the time of diagnosis was obtained by reviewing their case history.
JAK2V617F mutation was assessed in all patients by a tetra-primer PCR assay, as previously described. [18] Patients with PV and negative JAK2V617F were sequenced to screen for JAK2 exon 12 mutation [7]. Patients with non-mutated JAK2 PMF or ET were further evaluated for MPL exon 10 mutations. An allele-specific PCR assay was designed to detect MPL W515L and W515K. In three separate reactions, a 346-bp fragment was amplified with the following primers: W515 forward specific primer CTGCTGCTGCT GAGGT, LS15 forward specific primer CTGCTGCTGCTG AGGTT, KS15 forward specific primer CTGCTGCTGCTG AGGAA, and the common reverse primer AGGAGGT GGGACTGACG. Each reaction was performed from 50 to 200 ng of DNA in a final volume of 25 μl containing 0.4 μmol/L of each primer (Operon), dNTPs 0.2 mM (Promega), Cl2Mg 2.5 mM (Promega), Taq 1× buffer (Promega) and 1 U of DNA Taq polymerase (Promega). The PCR used the following cycle protocol: an initial denaturation at 95°C for 5 minutes was followed by 30 cycles at 95°C for 30 seconds, 60°C for 60 seconds and 72°C for 45 seconds, with a final elongation cycle at 72°C for 3 minutes. This assay was further validated by Sanger sequencing to determine the reliability of the method in a routine clinical setting (data not shown). In order to detect insertions or deletions in the exon 9 of CALR gene in patients with non-mutated JAK2 and MPL, the complete sequence of this exon was amplified by PCR and sequenced as previously described [16].

All the laboratory parameters included in the statistical analyses were gathered at diagnosis. Differences in the distribution of continuous variables between categories were analysed by either Mann–Whitney or Kruskal–Wallis tests. Patient groups with nominal variables were compared by the Chi-square test or Fisher’s exact test, when appropriate. All P-values were considered statistically significant when smaller than 0.05 (two-tailed). Statistical analyses were performed using the GraphPad Prism software, version 5.0 (GraphPad software, Inc., San Diego, CA, USA). Numerical variables have been summarized by their median and range, and categorical variables by count and relative frequency (%) of each category.

**Results**

As reported in Table 1, out of 439 patients studied, 329 (74.9%) carried JAK2V617F, 54 (12.3%) a CALR exon 9 frameshift mutation, 9 (2.1%) a MPL mutation, and 47 (10.7%) had non-mutated JAK2, MPL and CALR (i.e. triple-negative patients). We did not find any JAK2 exon 12 mutations. The positivity of the JAK2V617F mutation was 94.9% in PV, 61.2% in ET and 62% in PMF. MPL exon 10 mutations were found in 6% of ET and 2% of PMF. CALR exon 9 mutations were detected in ET and PMF, with mutational frequencies of 21.5 and 18%, respectively.

Out of the nine subjects with MPL mutations, seven carried W515L (five ET and two PMF subjects) and two carried W515K mutation (one ET and one PMF subject). Among subjects with CALR mutations, type 1 (c.1092_1143del) and type 2 (c.1154_1155insTTGTC) occurred in 20 cases (37%) and 24 cases (44.4%), respectively. In the remaining 10 cases (18.6%), seven variant mutations were found: c1095_1140del (4 cases), c.1118_1136del (one case), c.1125_1146del (one case), as well as four cases of previously unrecognized mutations (c.1094_1130del, c.1112_1154del, c.1127_1134delinsTCTTGGCTA and c.1149_1154delins TCTTCTTGGTC). In ET, type 1 was found in 19 cases (41.3%), type 2 in 17 (37%) and the other seven types were present in 10 cases (21.7%), while in PMF, type 1 was found in 1 case (12.5%) and type 2 in 7 (87.5%) (Table 1).

Table 2 reports the main clinical and haematological features at diagnosis of the ET patients studied according to their genotype. The MPL group was excluded from the statistical analysis due to its small size. CALR frameshift mutations were associated with
lower white blood cell counts, lower haemoglobin levels, higher platelet counts and lower age compared with JAK2 mutations. Triple-negative patients showed lower age at presentation, lower white blood cell counts, lower haemoglobin levels compared with patients with JAK2 mutations and had lower platelet counts than those with CALR mutations. In PMF, patients with mutant CALR were associated with higher platelet counts (Table 3).

Discussion

In this study, we found JAK2, MPL or CALR mutations in 94.9% of the cases with PV, 85.5% in patients with ET and 85.2% with PMF. Hence, the combined genetic tests of these driver mutations are essential for accurate diagnoses of BCR-ABL1-negative MPN. JAK2V617F mutation was the most frequent in the 3 subtypes of MPN studied followed by CALR mutations in ET and PMF. Among those patients without JAK2 or MPL mutations, the frequency of CALR frameshift mutations was 59.7% in ET and 53.3% in PMF, these findings being consistent with previous reports [15–16].

Nine different types of CALR exon 9 mutations were identified including deletions, insertions and complex indels. 81.4% of patients carried the most frequently described type 1 or 2 and the remaining 18.6% carried seven other types, four of which were novel. All novel mutations resulted in a common +1 bp-altered reading frame and predicted a novel C-terminal peptide sequence lacking the KDEL motif, as is the case for other mutations. Patients with PMF were limited to types 1 and 2, type 2 mutation (seven cases) being more frequent than the type 1 mutation (one case).

Although type 1 mutation was mainly associated with a myelofibrosis phenotype, [16,19–21] we found a significantly higher frequency in ET than in PMF (41.3 vs. 12.5%), as also observed in Chinese patients [22,23]. This may be attributed to the differences in the diagnostic criteria and ethnic origin.

When comparing the clinical and haematological characteristics of patients with ET, it was observed that patients with CALR exon 9 mutations were statistically younger than those with JAK2V617F. Triple-negative patients showed a tendency to be the youngest though there was no statistical significance between these patients and those with CALR mutations. In addition, JAK2V617F patients had high leucocyte and haemoglobin values compared to CALR-mutated and triple-negative patients. CALR-mutated patients had high platelet counts compared with JAK2V617F and triple-negative patients. Contrary to other studies, [24–26] we did not find a male predilection in CALR-mutated group. In the case of patients with PMF, it has been reported that CALR mutations are correlated with younger age, higher platelet count, lower leucocyte count and higher haemoglobin level [19,27]. We found that CALR patients had higher platelet count than those with JAK2V617F and triple-negative patients, although there was no statistical significance between CALR-mutated and JAK2V617F patients. In addition, JAK2V617F patients showed a tendency to high leucocyte and haemoglobin values compared to CALR-mutated and triple-negative patients. We also observed a male predilection and a tendency to be younger in triple-negative patients, but this should be confirmed by further studies with higher number of patients.

In conclusion, our findings indicate that the mutation profile of our BCR-ABL1-negative MPN patients is in line with previous reports. In addition, our data show similarities with these reports regarding clinical and haematological features of ET patients. However, we observed differences in the ratio of CALR mutation types and in clinical and haematological features in PMF. Probably, these discrepancies were due to the small size of CALR-mutated and triple-negative PMF subgroups. Finally, our study underscores the importance of JAK2, MPL and CALR genotyping for an accurate diagnosis of patients with BCR-ABL1-negative MPN.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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