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Letter to the Editor

Somatic mutations of calreticulin in a Brazilian cohort of patients with myeloproliferative neoplasms



Dear Editor,

Essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF) are Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) characterized by increased myeloid proliferation. The gain of function induced by the *Janus kinase 2* mutation, $JAK2^{V617F}$, has been reported in most PV and in more than half of ET and PMF cases.¹ However, the presence of different disease phenotypes and the absence of the $JAK2$ mutation in some MPNs suggests that additional genetic lesions or/and aberrant signaling pathways may be involved in the pathogenesis of these diseases.^{1,2}

In December 2013, somatic mutations in the *calreticulin* (*CALR*) gene were identified in ET and PMF patients by two independent groups^{3,4} and confirmed by others.^{5–9} *CALR* mutations have been reported as mutually exclusive with $JAK2$ and *MPL* mutations and may be present in 56–88% of $JAK2$ /*MPL*-negative cases.^{3,4} A recent paper reported a patient that had both mutations, $JAK2^{V617F}$ and a *CALR* exon 9 mutation, simultaneously.¹⁰ Over thirty different *CALR* mutations in exon 9 have been described, but the most frequent mutations (about 80%) may be classified as type-1 [L367fs*46; deletion of 52 base pairs (bp)] and type-2 (K385fs*47; insertion of 5 bp).^{3,4} The functional changes induced by the mutations are still not completely elucidated, but the overexpression of the type 1 *CALR* mutation in Ba/F3 cells (an IL3 dependent cell line) leads to cytokine-independent cell growth and STAT5 activation.⁴ Gene expression signature studies also indicate that $JAK2$ and *CALR* mutations share mechanisms of malignant transformation, reaffirming a central role of the *JAK*/*STAT* signaling pathway in the pathogenesis of MPN.¹¹

The aim of the present study was to characterize the prevalence of *CALR* mutations and the clinical and laboratorial characteristics of *CALR*-mutated patients in a Brazilian cohort of MPNs. Seventy-three MPN patients were included in the study (ET = 32, PV = 20, PMF = 21). Patients' characteristics are described in Table 1. Peripheral blood samples were collected, submitted to hemolysis, and DNA was extracted by the

phenol/chloroform method. All samples were investigated for $JAK2$ and *CALR* mutations. The $JAK2^{V617F}$ mutation and *CALR* exon 9 mutations were verified as previously described.^{12,13} *CALR* mutations were classified as type-1 (deletion of 52 bp), type-2 (insertion of 5 bp) or others (Figure 1).

In our cohort, *CALR* mutations were found in 20 patients (13 ET and 7 PMF; Table 1) and were mutually exclusive with $JAK2^{V617F}$: 20/73 (27%) of total MPN patients and 20/28 (71%) of the $JAK2^{WT}$ patients. Among the *CALR*-mutated patients, Type-1 *CALR* mutations were found in 50% (10/20; 8 ET and 2 PMF), type-2 in 40% (8/20; 4 ET and 4 PMF), and others in 10% (1 ET and 1 PMF) of the individuals. *CALR* mutations were not detected in PV patients (all $JAK2^{V617F}$ positive). In ET, *CALR*^{MUT} patients showed reduced hemoglobin levels compared with $JAK2^{V617F}$ patients (*p*-value <0.01; Table 2); no differences were observed in white blood cell, neutrophil and platelet counts, thrombotic events, hepatomegaly, splenomegaly and constitutional symptoms. In PMF patients, *CALR* mutational status was not associated with clinical features (Table 2). The frequency of *CALR* mutations in this Brazilian cohort was similar to previously described frequencies.^{3,4,7} Furthermore, *CALR* and $JAK2^{V617F}$ mutations were mutually exclusive. Knowledge regarding the clinical impact of *CALR* mutations in MPNs is still under construction, but some studies indicate that *CALR*-mutated patients have lower ages at disease onset, lower hemoglobin and platelet counts, and have better overall survival than either $JAK2$ -mutated or *CALR*/ $JAK2$ /*MPL* wild-type patients.^{5,9,14} In our cohort, *CALR*^{MUT} ET patients presented lower hemoglobin levels, compared with $JAK2^{V617F}$ ET patients, even though in both groups hemoglobin values remained within the reference range.

In conclusion, *CALR* mutations are highly frequent in Brazilian patients with MPN. The search for *CALR* mutations may be a useful tool for MPN diagnosis and further research on this mutation in Brazilian patients would be important to define the local incidence of the mutation, to improve diagnosis and classification of our patients, and to better evaluate the impact of these mutations on the outcomes of MPN patients.

Table 1 – Patient characteristics.

	MPN (n = 73)	ET (n = 32)	PV (n = 20)	PMF (n = 21)
Age (years) – median (range):	57 (19–87)	53 (19–83)	70 (41–88)	62 (27–87)
Gender – male/female	30/43	10/22	9/11	11/10
Hemoglobin (g/dL) – median (range)	13.9 (8.1–22.7)	13.6 (9.7–17)	18.4 (12.8–22.7)	12.5 (8.1–19.9)
WBC count ($\times 10^9 \text{ L}^{-1}$) – median (range)	9.8 (2.4–43.2)	9 (3.0–17.8)	11.2 (6.3–25.5)	11.8 (2.4–43.2)
Platelet count ($\times 10^9 \text{ L}^{-1}$) – median (range)	764 (108–2065)	914 (541–2065)	609 (203–1070)	661 (108–1716)
JAK2 mutation status – n (%)				
JAK2 ^{WT}	28 (38)	20 (63)	0 (0)	8 (38)
JAK2 ^{V617F}	45 (62)	12 (37)	20 (100)	13 (62)
CALR mutation status – n (%)				
CALR ^{WT}	53 (73)	19 (59)	20 (100)	14 (67)
CALR ^{MUT}	20 (27)	13 (41)	0 (0)	7 (33)

MPN: myeloproliferative neoplasms; ET: essential thrombocythemia; PV: polycythemia vera; PMF: primary myelofibrosis; WBC: white blood cell count; JAK2: Janus kinase 2 gene; WT: wild-type; CALR: Calreticulin gene; MUT: exon 9 mutations.

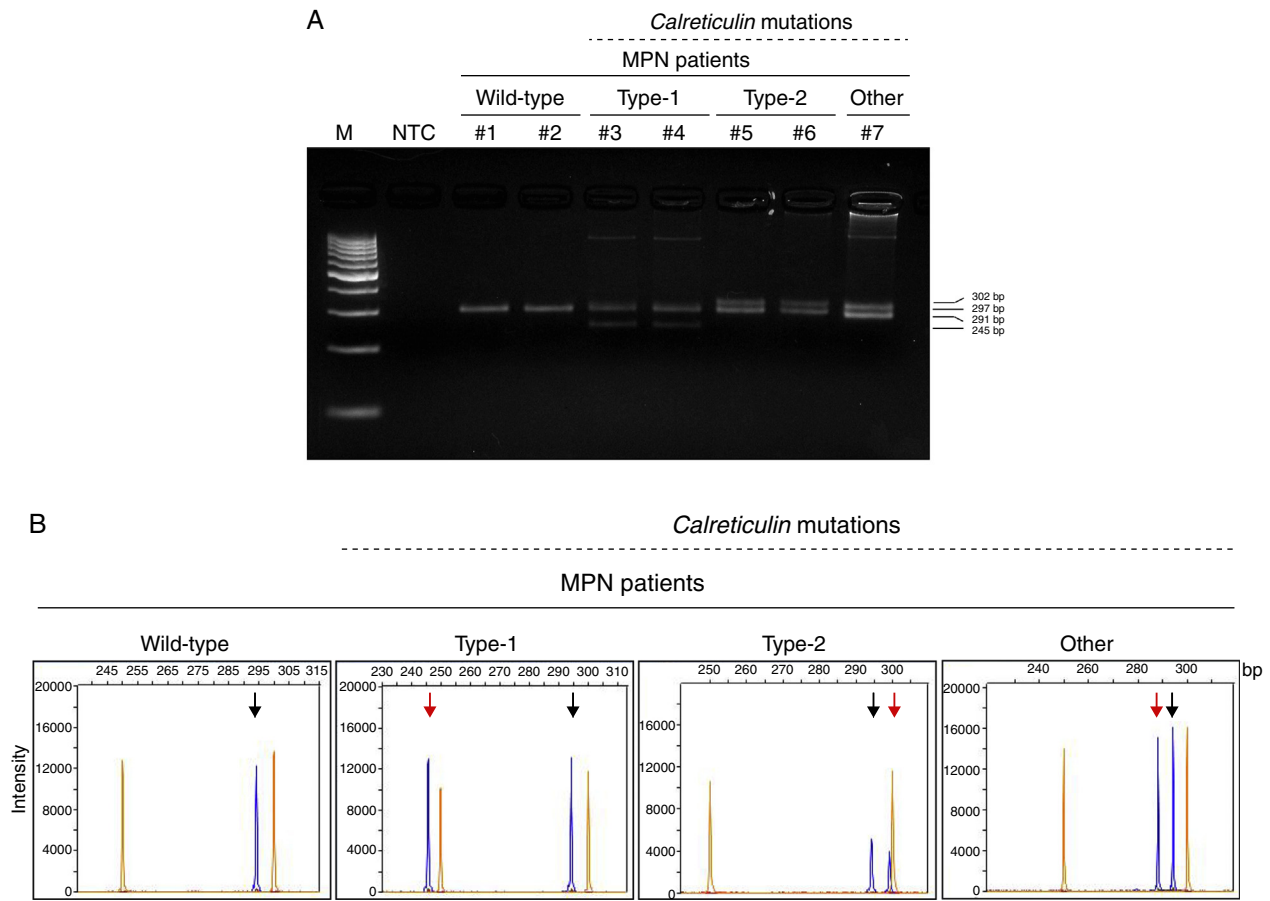


Figure 1 – Calreticulin (CALR) indel mutations in myeloproliferative neoplasm (MPN) patients. (A) PCR amplification of exon 9 of CALR gene loaded on 4% agarose gel; lane 1: 100 bp marker (M); lane 2: no template control (NTC); MPN patients #1 and #2: CALR^{WT}; #3 and #4: heterozygotes for type-1 CALR mutation (CALR^{WT} amplicons: 297 bp; type-1 CALR^{MUT} amplicons: 245 bp); #5 and #6: heterozygotes for type-2 CALR mutation (CALR^{WT} amplicons: 297; type-2 CALR^{MUT} amplicons: 302 bp); #7: heterozygote for non-type-1 or -2 CALR mutation (CALR^{WT} amplicons: 297 bp; CALR^{MUT} amplicons: 291 bp). **(B)** Representative PCR fragment size analysis of CALR amplicons from MPN patients: the orange peaks represent the GeneScan 500 LIZ dye Size Standard and blue peaks represent the CALR amplicons; the black arrows indicate the wild-type allele (297 bp) and the red arrows indicate the indel-mutation alleles.

Table 2 – Clinical and laboratory features of essential thrombocythemia and primary myelofibrosis patients, stratified according to JAK2 and CALR mutational status.

	Essential thrombocythemia				Primary myelofibrosis ^b			
	CALR ^{MUT}	JAK2 ^{V617F}	CALR ^{WT} /JAK2 ^{WT}	p-value ^{a,c}	p-value ^{a,d}	CALR ^{MUT}	JAK2 ^{V617F}	p-value ^{a,c}
Gender: male/female – n (%)	4 (31)/9 (69)	5 (42)/7 (58)	1 (14)/6 (86)	0.69	0.61	4 (57)/3 (43)	6 (46)/7 (54)	1.00
Age – years (range)	57 (20–82)	55 (44–83)	43 (19–53)	0.61	0.13	53 (27–80)	65 (43–87)	0.30
Hemoglobin – g/L (range)	13 (10–15)	14 (12–17)	14 (12–16)	0.007	0.12	12 (9–13)	14 (8–20)	0.08
White blood cells – $\times 10^9/L^{-1}$ (range)	7.1 (3–17.8)	9.2 (4.5–16.7)	9 (5–11.8)	0.64	0.69	9.4 (2.4–35.7)	11.8 (8.4–43.2)	0.38
Neutrophils – $\times 10^9/L$ (range)	4.8 (2.1–15.1)	6 (2.5–12)	5.6 (3.2–8.5)	0.43	0.48	6.2 (1–22.5)	7.1 (0.5–38.3)	0.48
Platelets – $\times 10^9/L$ (range)	938 (593–2065)	880 (541–1340)	984 (617–1274)	0.37	0.87	567 (108–1716)	661 (129–1606)	0.69
Thrombotic events – n (%)	0 (0)	2 (17)	0 (0)	0.50	1.00	0 (0)	1 (8)	1.00
Hepatomegaly – n (%)	1 (8)	0 (0)	0 (0)	1.00	1.00	0 (0)	2 (17)	1.00
Splenomegaly – n (%)	2 (15)	1 (8)	1 (14)	1.00	1.00	5 (71)	7 (54)	0.64
Constitutional symptoms – n (%)	1 (8)	3 (25)	2 (29)	0.32	0.27	1 (14)	3 (23)	1.00

^a Fisher's exact test was used for categorical factors with 2 levels; Mann-Whitney test for measured factors.^b Only one patient with primary myelofibrosis presented CALR^{WT}/JAK2^{WT}.^c CALR^{MUT} vs. JAK2^{V617F}.^d CALR^{MUT} vs. CALR^{WT}/JAK2^{WT}.

Conflicts of interest

The authors declare no conflicts of interest.

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