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## ORIGINAL RESEARCH REPORT

# Mutation Profile in *BCR-ABL1*-Negative Myeloproliferative Neoplasms: A Single-Center Experience From India

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## Abstract

**Objective/Background:** Recurrent somatic mutations in the *JAK2*, calreticulin (*CALR*), and the *MPL* genes are described as drivers of *BCR-ABL1*-negative myeloproliferative neoplasms (MPN) that includes polycythemia vera (PV), essential thrombocytosis (ET), primary myelofibrosis (PMF), and MPN unclassified (MPN-U).

**Methods:** We describe the mutation profile and clinical features of MPN cases diagnosed at a tertiary care center. *JAK2V617F* and *MPL* (S505/W515) mutations were screened by allele-specific polymerase chain reaction, while *CALR* exon 9 and *JAK2* exon 12 mutations were screened by fragment analysis/Sanger sequencing. Among the 1,570 patients tested for these mutations during the study period, 407 were classified as MPN with a diagnosis of PV, ET, PMF, and MPN-U seen in 30%, 17%, 36%, and 17%, respectively, screened.

**Results:** Similar to previous reports from Asian countries, the incidence of PMF was the highest among the classic MPN. *JAK2V617F* mutation was detected in 90% of PV, 38% of ET, 48% of PMF, and 65% of MPN-U. *JAK2* exon 12 mutations were seen in 5.7% of PV and 1.4% of PMF. *CALR* exon 9 mutations were seen in 33% of ET, 33% of PMF, and 12% of MPN-U. *MPL* mutations were detected in 2.8%, 2.7%, and 2.9% of ET, PMF, and MPN-U, respectively. Fifteen % of PMF, 26% of ET, and 22% of MPN-U were triple negative.

**Conclusion:** There was a significantly higher incidence of *CALR* mutation in PMF and ET cases. Our study highlights the challenges in the diagnosis of *JAK2*-negative PV and the need for harmonization of criteria for the same.

**Keywords:** *CALR*, India, *JAK2*, *MPL*, Myeloproliferative neoplasm, Mutations

## 1. Introduction

The classical myeloproliferative neoplasms (MPNs), also called the *BCR-ABL1*-negative MPNs, are a group of related clonal hematologic disorders characterized by excess accumulation of one or more myeloid cell lineages. Polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF) are categorized as classic MPN [1]. They are clinically characterized by

nonspecific symptoms such as fatigability, pruritus, early satiety due to splenomegaly, increased risk of infections, and thrombotic events [2]. Disease morbidity is associated with thromboembolic and hemorrhagic events. The complication of the disease by transformation to myelofibrosis is seen to a greater extent with PV than ET and to secondary AML with PMF than with PV or ET [2,3].

In the vast majority of MPN cases, a mutation in codon 617 of *JAK2*, resulting in the replacement of

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the amino acid valine with phenylalanine [V617F, *JAK2* NM\_004972.3:c.1849G > T (p.Val617Phe)] is found. This gain of function mutation is present in approximately 96% of PV, 55% of ET, and 65% of PMF [4]. *JAK2* exon 12 mutations are described in 5% of cases of PV [4] and in PMF [5]. Point mutations in codon 515 of the thrombopoietin receptor gene (*MPL*) have been reported in 5–10% of cases of *JAK2*-negative ET and PMF. Mutations in the calreticulin (*CALR*) gene have been reported in ~20–25% of ET and PMF [6].

Dysregulated *JAK2* signaling is described as the central phenotypic driver of *BCR-ABL1*-negative MPNs. Furthermore, MPNs exhibit unexpected levels of genetic complexity, with multiple abnormalities associated with disease progression, interactions between hereditary factors and driver mutations, and effects related to the order in which the mutations are acquired during the disease process [1,6,7].

Although morphology and clinical laboratory analysis continue to play an important role in defining these conditions, emphasis on molecular testing in MPN is made by the World Health Organization (WHO) by including *JAK2*, *MPL*, and *CALR* mutations as one of the diagnostic criteria in the 2016 update on the classification of myeloid neoplasms [8]. Genomic analysis is being increasingly recognized to be of significance in prognostication in addition to diagnosis.

Most of the available data [9–11] regarding the incidence, prevalence, distribution of subtypes, molecular patterns, and natural history come from the developed world. There is limited data from low- and middle-income countries where challenges remain in diagnosing these conditions. The population pyramid is skewed to a younger age; therapeutic interventions are inadequate and often delayed. The spectrum and profile of classical MPNs presenting to a tertiary center in such low- and middle-income settings are likely to be significantly different than is conventionally expected [9]. There is a paucity of large data sets describing the mutation spectrum of MPN from tertiary care centers in India. Here, we describe the pattern of mutations and clinical features in patients with MPN at our center.

## 2. Patients and methods

### 2.1. Patients

All patients tested in our laboratory for *JAK2* V617F, *JAK2* exon 12, *CALR*, and *MPL* hotspot mutations (MPN panel) between January 2016 and

March 2020 were included in this study. Clinical and laboratory characteristics at diagnosis or referral were documented by reviewing the patients' electronic records. A multidisciplinary team reviewed the available clinical, laboratory, and histopathological information to make the diagnosis, and they were subsequently classified as MPN based on the WHO 2016 criteria. Cases that did not meet these criteria and those with inadequate data were excluded from further analysis. Chronic neutrophilic leukemia, chronic eosinophilic leukemia, and myelodysplastic syndrome/MPN were also excluded from this analysis. Stringent criteria (British Society for Haematology [BSH] 2019) [12] were applied to classify cases of *JAK2*-negative PV (Supplementary Table S1).

### 2.2. Screening of *JAK2*, *CALR*, and *MPL* mutations

DNA was extracted from whole blood by Gentra Puregene, blood DNA kit (Qiagen, Hilden, Germany). DNA quality and quantity of these samples were assessed by Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). *JAK2* V617F, *CALR*, *MPL*, and *JAK2* exon 12 mutations were screened in all the samples that were referred for MPN panel analysis. *JAK2* V617F mutation was screened by an allele-specific polymerase chain reaction assay (assay sensitivity 1%), as reported previously [4]. *JAK2* exon12 mutations was screened by Sanger sequencing, while mutations in *CALR* exon 9 were screened using capillary electrophoresis-based fragment length analysis as reported previously [4,13]. The type of *CALR* mutation was identified by Sanger sequencing. Four common mutations in *MPL* were screened using an allele-specific oligonucleotide polymerase chain reaction assay as reported previously [14].

### 2.3. Statistical analysis

The chi-square test and Mann–Whitney *U*-test were used to compare variables. For all the tests, a two-sided *p* value  $\leq 0.05$  was considered statistically significant. The analysis was done using IBM SPSS statistics version 24.0 software (SPSS, Chicago, IL, USA).

## 3. Results

During the study period, 1570 samples were screened for MPN panel (*JAK2*V617F, *JAK2* exon 12, *MPL*, and *CALR* mutations) with a suspected diagnosis of MPN (Fig. 1). Of these, 407 cases fulfilled the WHO 2016 criteria for MPN and were further

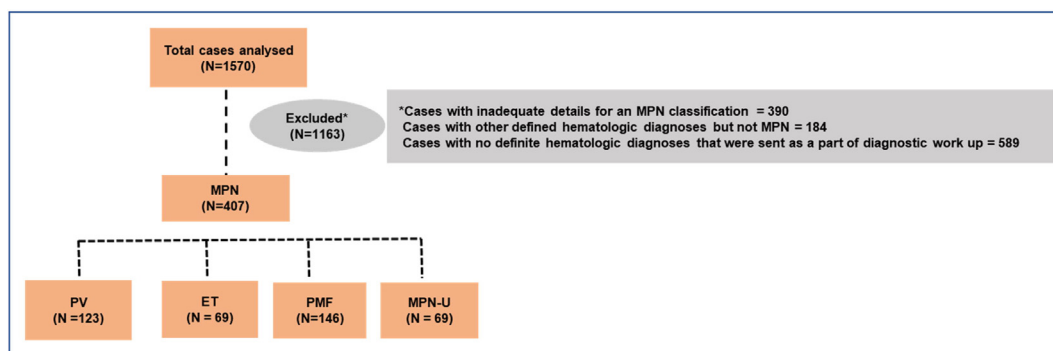


Fig. 1. Representation of MPN categorization among the cases analyzed. ET = essential thrombocytosis; MPN = myeloproliferative neoplasm; MPN-U = myeloproliferative neoplasm unclassified; PMF = primary myelofibrosis; PV = polycythemia vera.

analyzed. The demographics of the patients are listed in [Supplementary Table S2](#). The median age of this cohort was 50 years (range: 1–80 years) with a male predominance (M:F = 2.1:1). All MPN cases were subcategorized further into PV ( $n = 123$ ), ET ( $n = 69$ ), PMF ( $n = 146$ ), and MPN unclassified (MPN-U) ( $n = 69$ ). Laboratory features of individual subtypes are listed ([Supplementary Table S2](#)). *JAK2*, *MPL*, and *CALR* mutations were mutually exclusive of each other.

### 3.1. Mutation spectrum in PV

Among the 123 patients with PV, 118 were *JAK2* mutation-positive (V617F in 94% [ $n = 111$ ] and exon 12 mutations 6% [ $n = 7$ ]) ([Fig. 2A](#)). Of the seven patients with *JAK2* exon 12 mutations, one was a missense mutation and six were indels. These patients presented with absolute erythrocytosis, which is characteristic of *JAK2* exon 12 mutations. However, bone marrow morphology was similar to that of *JAK2*V617F-mutated PV with no isolated erythroid hyperplasia. Thrombotic events such as thrombosis of the portal vein, central vein, superior mesenteric vein, deep veins of the lower limbs and cardiovascular events (STEMI/ischemia) were recorded in 19.5% (24 of the 123 cases), while splenomegaly was seen in 34% (42 of the 123) cases.

### 3.2. Mutation spectrum in ET

The median age at diagnosis of the patients classified as having ET ( $n = 69$ ) was 36 years (range: 1–78). Mutations identified in the driver genes in this group were: *JAK2*V617F (37.6%), *CALR* (33.3%), and the *MPL* (2.8%) ([Fig. 2B](#)). Among the *JAK2*<sup>wt</sup>/*MPL*<sup>wt</sup> *CALR*-mutated cases, type 2 was more common (52%;  $n = 12$ ), followed by type 1 (44%;  $n = 10$ ) and a 34 base pair deletion was classified as type 1 like (c.1092\_1125del; p.E364Dfs\*55) (4%) ([Table 1](#)).

No mutation in any of the driver genes was identified in 26% ( $n = 18$ ) cases.

Comparison of demographics in patients with ET having different mutations ([Table 2](#)) showed a significantly higher platelet count and a lower total leucocyte count in the *CALR*-mutated ET than the *JAK2*-mutated ET. Patients who were categorized as triple-negative ET were significantly younger with lower hemoglobin and higher platelet counts when compared with patients with ET having a mutation in any of the three driver genes.

Thrombotic events were recorded in 13% ( $n = 9$ ) among the 69 cases of ET in this study. These included occlusions in the retinal artery, the coronary artery leading to ischemia, thrombosis of major cerebral vessels, deep vein thrombosis, and one case of extrahepatic portal vein obstruction. Splenomegaly was seen in 17.3% ( $n = 12$ ).

### 3.3. Mutation spectrum in PMF

Among the 146 cases classified as PMF, *JAK2*V617F, *CALR*, and the *MPL* mutations were seen in 48.3%, 32.9%, and 2.7% of them, respectively ([Fig. 2C](#)). Of the *JAK2*<sup>wt</sup>/*MPL*<sup>wt</sup> cases, six different types of *CALR* mutations were identified. The majority of these were type 1, (75%;  $n = 36$ ), 17% ( $n = 8$ ) were type 2, 6% ( $n = 3$ ) were type 1-like, and one was classified as other type (2%) ([Table 1](#)). A comparison of demographics in patients with PMF having different mutations ([Table 2](#)) showed that patients with *CALR*-mutated PMF had higher platelet counts than *JAK2*-mutated PMF. Patients who had no mutation in any of the driver genes were younger and had lower platelet counts and LDH values when compared with PMF with mutation in any of the driver genes.

A *CALR* mutation was detected in one case (c.1099\_1152delinsAG; p.L367Rfs\*46) which was novel in terms of a genomic change where there was

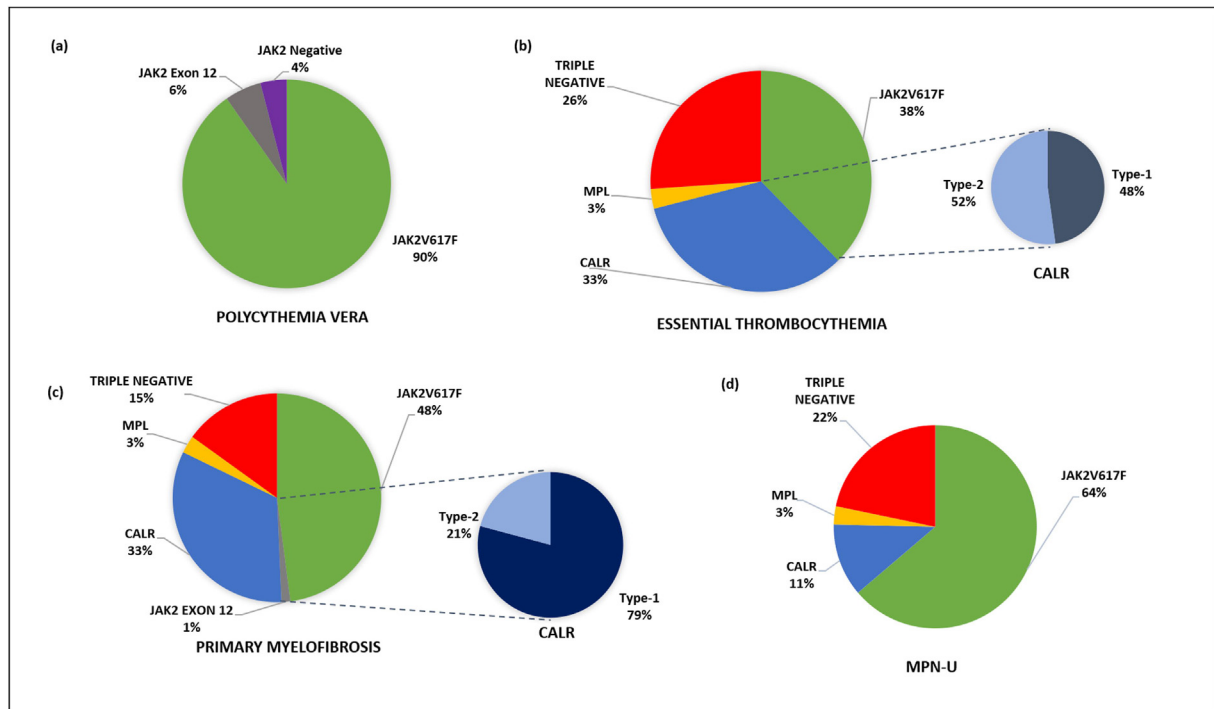


Fig. 2. Mutation profile of polycythemia vera. (A) Essential thrombocythemia; (B) primary myelofibrosis; (C) MPN-U; (D) in the present study. Note. CALR = calreticulin; MPL = myeloproliferative leukaemia virus oncogene; MPN-U = myeloproliferative neoplasm unclassified.

an indel (complex deletion insertion) involving an insertion of two nucleotides AG in the alternate allele along with a 54 base pair deletion. This molecular event, however, resulted in a protein change similar to the type 1 deletion except the first amino acid change is arginine instead of threonine. Hence, this was classified as type 1-like based on the predicted effect on three different stretches of negatively charged amino acids of the wild-type CALR sequence (Fig. 3). Tryptophan was replaced by leucine at codon 515 (c.1544G > T; p.W515L) in 75% ( $n = 3$ ) of the MPL-mutated cases and in one case the substituted amino acid was lysine (MPL:p.W515K). No mutation in any of the driver genes was identified in 15% ( $n = 22$ ) of cases.

Thrombotic events were recorded in only five patients with PMF. Splenomegaly ranging from tip palpable to massive splenomegaly (2–28 cm) was observed in 111 of 146 cases. Two cases underwent a splenectomy at the time of diagnosis.

### 3.4. MPN-U

Patients with overlapping features that could not be further categorized as any particular classical MPN ( $n = 69$ ) were placed under the MPN-U entity. JAK2V617F mutations were identified in 65% ( $n = 44$ ), CALR mutations in 11.6% ( $n = 8$ ) (seven with type 1 and one with type 2), and MPL mutation in 2.9% ( $n = 2$ ) (p.W515K) of these patients (Fig. 2D).

Table 1. Spectrum of Calreticulin (CALR) mutations identified in PMF, ET, and MPN-U in our study.

Mutation (CDS); amino acid <sup>a</sup>	Type of mutation	COSMIC mutation ID	Number of cases		
			PMF (N = 48)	ET (N = 23)	MPN-U (N = 8)
c.1099_1150del; p.L367Tfs*46	Type-1; 52 bp deletion	COSM1738055	75% (N = 36)	44% (N = 10)	87.5% (N = 7)
c.1154_1155insTTGTC; p.K385Nfs*47	Type-2; 5 bp insertion	COSM1738056	17% (N = 8)	52% (N = 12)	12.5% (N = 1)
c.1097_1130del; p.R366Kfs*53	Type-1 like; 34 bp deletion	COSM1738357	6% (N = 3)	—	—
c.1100_1145del; p.L367Qfs*48	Type-1 like; 46 bp deletion	COSM1738150	—	—	—
c.1099_1152delinsAG; p.L367Rfs*46	Type-1 like	Novel	—	—	—
c.1120_1125delinsTGCGT; p.K374Cfs*56	Other	COSM3355758	2% (N = 1)	—	—
c.1092_1125del; p.E364Dfs*55	Type-1 like; 34 bp deletion	COSM1738333	—	4% (N = 1)	—

Note. ET = essential thrombocythemia; MPN-U = myeloproliferative neoplasm unclassified; PMF = primary myelofibrosis.

<sup>a</sup> Transcript ID: CALR-201 ENST00000316448.10; RefSeq: NM\_004343.4 for all the mutations in the CALR gene described in this study.

Table 2. Laboratory characteristics of essential thrombocytosis (n = 69) and primary myelofibrosis (n = 146) patients according to mutation profiles in this study.

	N (%)	Age (yr)	M:F (% males)	Hemoglobin (g/dL)	Hematocrit	Total WBC count ( $\times 10^9/L$ )	Platelet count ( $\times 10^9/L$ )	LDH (U/L) <sup>a</sup>
ET (mutation-positive)	51 (74)	42 (12–78)	24:27 (47)	12.9 (8.9–16)	39.6 (27.5–47.5)	10.2 (5.2–55)	875 (462–2,368)	622 (338–3820)
ET (mutation-positive)								
<i>JAK2</i>	26 (37.6)	46 (12–78)	11:15 (42.3)	12.8 (10.1–15.7)	39.8 (31.3–47.5)	11.3 (5.2–36.3)	779 (462–1,856)	601.5 (401–3820)
<i>CALR</i>	23 (33.3)	37 (24–65)	12:11 (52)	12.9 (8.9–16)	39.2 (27.5–46.4)	9.6 (5.8–55)	1,045 (657–2,368)	629 (423–1589)
<i>MPL</i>	2 (2.9)	(66, 62)	01:01	13.1	39.1 (38.7–39.6)	6.7 (5.4–8.1)	716 (672–760)	569
Triple-negative ET (Mutated vs. triple-negative)	18 (26)	18 (1–60)	7:11 (38.8)	11.9 (9.5–14.3)	35.6 (29.5–43.5)	10.2 (4.4–15.4)	1,190 (622–2,708)	618 (338–1,117)
<i>p</i> value*	—	<b>0.002</b>	0.54	<b>0.001</b>	<b>0.0002</b>	0.58	<b>0.02</b>	0.8
( <i>JAK2</i> V617F vs. <i>CALR</i> )	—	0.42	0.48	0.91	0.25	0.02	0.01	0.56
<i>p</i> value*	—	<b>0.002</b>	0.54	<b>0.01</b>	<b>0.009</b>	0.09	0.32	0.8
( <i>CALR</i> vs. triple-negative)	—	<b>0.002</b>	0.54	<b>0.01</b>	<b>0.009</b>	0.09	0.32	0.8
<i>p</i> value*	—	<b>0.001</b>	0.82	<b>0.004</b>	<b>0.0001</b>	0.36	<b>0.004</b>	0.98
( <i>JAK2</i> vs. triple-negative)	—	<b>0.001</b>	0.82	<b>0.004</b>	<b>0.0001</b>	0.36	<b>0.004</b>	0.98
<i>p</i> value*	—	<b>0.001</b>	0.82	<b>0.004</b>	<b>0.0001</b>	0.36	<b>0.004</b>	0.98
PMF (mutation-positive)	124 (85)	54 (31–80)	87:38 (69.6)	9.6 (3.7–15.8)	29.9 (11.6–54.2)	8.7 (1.8–197.4)	243 (7–1664)	1,162 (403–3,896)
PMF (mutation-positive)								
<i>JAK2</i>	70 + 2 <sup>b</sup> (47.9 + 1.4)	55 (36–80)	47:23 (67)	9.85 (3.8–15.8)	31.3 (11.6–54.2)	11.5 (1.8–152.5)	211.5 (7–1664)	1,144 (403–3,687)
<i>CALR</i>	48 (32.8)	50 (31–76)	36:12 (75)	10.1 (3.7–14.7)	29.9 (11.7–44.4)	7.85 (2.5–197.4)	295.5 (9–1565)	1,235 (562–3,896)
<i>MPL</i>	4 (2.7)	61 (51–74)	02:02	8 (7.6–9.2)	25.7 (24–28.4)	5.35 (4.2–21.7)	104.5 (63–342)	1,490.5 (1,157–2,700)
Triple-negative PMF (Mutated vs. triple-negative)	22 (15)	42 (14–64)	16:6 (72.7)	8.4 (5.9–14.7)	27.9 (18.3–42.5)	7 (1.3–66.5)	87.5 (7–1371)	723.5 (241–1803)
<i>p</i> value*	—	<b>0.002</b>	0.76	0.07	0.17	0.41	<b>0.02</b>	<b>0.001</b>
( <i>JAK2</i> V617F vs. <i>CALR</i> )	—	0.013	0.84	0.81	0.66	0.07	0.01	0.24
<i>p</i> value*	—	0.013	0.84	0.81	0.66	0.07	0.01	0.24
( <i>CALR</i> vs. triple-negative)	—	<b>0.026</b>	0.83	0.95	0.94	0.42	0.68	<b>0.002</b>
<i>p</i> value*	—	<b>0.026</b>	0.83	0.95	0.94	0.42	0.68	<b>0.002</b>
( <i>JAK2</i> vs. triple-negative)	—	< <b>0.00001</b>	0.62	0.09	0.17	0.16	0.1	<b>0.006</b>
<i>p</i> value*	—	< <b>0.00001</b>	0.62	0.09	0.17	0.16	0.1	<b>0.006</b>

Note. *CALR* = calreticulin; ET = essential thrombocytosis; LDH = lactate dehydrogenase; *MPL* = myeloproliferative leukaemia virus oncogene; PMF = primary myelofibrosis; WBC = white blood cell.

\* All *p* values were calculated using the  $\chi^2$  test for categorical variables and the Mann–Whitney *U* test for continuous variables. Significant values are in boldface. <sup>a</sup>- Only cases with documented LDH values were included for analysis in the table [For ET: *JAK2* positive (n=16); *CALR* positive (n=19); *MPL* positive (n=1) and Triple negative (n=13)]. [For PMF with mutation (n=105); *JAK2* (n=62); *CALR* (n=39); *MPL* (n=4); TN-PMF (n=20)]. <sup>b</sup>- patients with *JAK2* exon12 mutations

AA Change:	Protein sequence	
	I            II            III	
REFERENCE :	QDEEQRLKKEE EEDKKRKEEE EAEKEDDED KEEDEDEED KEEDEEEDVP GQAKDEL	
1.p.L367Tfs*46:	QDEEQRLKKEE EEDKKRKEEE EAEKEDDED KEEDEDEED KEEDEEEDVP GQAKDEL	TYPE-1: STRETCH II & III DELETED
2.p.L367Qfs*48:	QDEEQRLKKEE EEDKKRKEEE EAEKEDDED KEEDEDEED KEEDEEEDVP GQAKDEL	TYPE-1 LIKE: STRETCH II & III DELETED
3.p.L367Rfs*46:	QDEEQRLKKEE EEDKKRKEEE EAEKEDDED KEEDEDEED KEEDEEEDVP GQAKDEL	TYPE-1: STRETCH II & III DELETED
4.p.E364Dfs*55:	QDEEQRLKKEE EEDKKRKEEE EAEKEDDED KEEDEDEED KEEDEEEDVP GQAKDEL	TYPE-1 LIKE: STRETCH II & III DELETED
5.p.R366Kfs*53:	QDEEQRLKKEE EEDKKRKEEE EAEKEDDED KEEDEDEED KEEDEEEDVP GQAKDEL	TYPE-1 LIKE: STRETCH II & III DELETED
6.p.K374Cfs*56:	QDEEQRLKKEE EEDCVAKRRR RQRTRMMRT KMRMRMRRT RRMRRKMSA ARPRTSCREA CLQGWTEA*	OTHER TYPE: STRETCH III DELETED
7.p.K385Nfs*47:	QDEEQRLKKEE EEDKKRKEEE EAEKEDDED KEEDEDEED KEEDEEEDVP GQAKDEL	TYPE II: STRETCH I,II,III MAINTAINED

Fig. 3. Alignment of C-domain in wild-type and mutant calreticulin (CALR) proteins. The reference amino acid sequence starts from codon A361: acidic, basic, and neutral residues are in red, blue, and green, respectively. All the variants involved three different stretches of negatively charged amino acids, here defined as I, II, and III, and highlighted in red in the wild-type sequence. Type 1-like mutations predict deletion of stretches II and III (as happens with the L367fs\*46 or type 1 mutation), while type 2-like mutations predict conservation of all three stretches (as happens with the K385fs\*47 or type 2 mutation); other types involve deletion of stretches III exclusively.

No mutations in any of the driver genes were identified in 21.7% ( $n = 15$ ) of these cases. Both arterial and venous thrombotic events were recorded in 24.6% ( $n = 17$ ) cases. Splenomegaly was observed in 49% (34/69) cases.

#### 4. Discussion

The discovery of mutations in the three driver genes (*JAK2*, *CALR*, and *MPL*) has significantly contributed to the understanding of the classical *BCR-ABL1*-negative MPN. The introduction of mutations in one of the driver genes as a major WHO criterion highlights the importance of testing for these markers in the diagnosis, classification, and prognosis of various MPN subtypes. Our study is one of the largest single-center studies on MPN from India, where we report the proportion of MPN

patients with PV, ET, PMF, and MPN-U of 30.2%, 17%, 35.8%, and 17%, respectively. When compared with the registry data from other Asian countries, the frequency of PMF was higher and ET was lower in our study group [9].

*JAK2V617F* mutation accounts for ~95% of PV and mutation in exon 12 contributes to a further 2–3% of these cases. Classification of cases presenting with persistent and significant erythrocytosis which are *JAK2*-negative is difficult and are classified as ‘*JAK2*-negative PV’ only after excluding all possible causes of secondary erythrocytosis. Stringent criteria for this entity, as laid out by the BSH 2019 guidelines, aid in differentiating this rare entity from idiopathic and secondary erythrocytosis. In this study, *JAK2V617F* accounted for 90.3% and exon 12 mutations, 5.7% of PV.

Table 3. Comparison of frequencies of driver mutations in myeloproliferative neoplasms.

		Median age (range), yr	<i>JAK2</i> V617F (%)	<i>JAK2</i> Exon 12 (%)	<i>JAK2</i> -negative (%)	<i>CALR</i> (%)	<i>MPL</i> (%)	Triple-negative (%)
PV	Kim et al. ( $N = 58$ ) [25]	57.4 (25.8–75.9)	87.9	3.5	8.6	—	—	—
	Pardanani et al. ( $N = 220$ ) [26]	—	97	2	1	—	—	—
	Passamonti et al. ( $N = 338$ ) [15]	—	95	4	1	—	—	—
	Ancochea et al. ( $N = 99$ ) [27]	69 (20–94)	94	3	3	—	—	—
	Rabade et al. ( $N = 20$ ) [28]	50 (31–83)	100	—	—	—	—	—
	Present study ( $N = 123$ )	51 (21–75)	90.3	5.7	4	—	—	—
ET	Kim et al. ( $N = 79$ ) [25]	55.0 (19–84)	63.3	—	—	17.7	2.5	16.5
	Tefferi et al. ( $N = 299$ ) [18]	56 (15–91)	53	—	—	32	3	12
	Rotunno et al. ( $N = 576$ ) [29]	58.1 (13–93)	64.1	—	—	15.5	4.3	16.1
	Rabade et al. ( $N = 34$ ) [28]	46 (21–76)	61.7	—	—	15.1	9.1	15.2
	Present study ( $N = 69$ )	36 (1–78)	37.8	—	—	33.3	2.9	26
PMF	Kim et al. ( $N = 54$ ) [25]	62 (8–83)	57.4	—	—	14.8	9.3	20.4
	Tefferi et al. ( $N = 254$ ) [19]	64 (32–87)	58	—	—	25	8.3	8.7
	Sazawal et al. ( $N = 80$ ) [30]	48 (32–63)	56.2	—	—	11.2	0	32.6
	Rabade et al. ( $N = 59$ ) [28]	53 (16–81)	57.6	—	—	23.7	3.4	15.3
	Present study ( $N = 146$ )	52 (14–80)	48	1.4	—	32.9	2.7	15

Note. *CALR* = calreticulin; ET = essential thrombocytosis; *MPL* = myeloproliferative leukaemia virus oncogene; PMF = primary myelofibrosis; PV = polycythemia vera.



Confirming the diagnosis of *JAK2*-negative PV has been a challenge. Although 52 cases in this study were categorized as *JAK2*-negative PV based on the WHO 2016 criteria for PV, only five of these cases met the BSH 2019 criteria. The WHO criteria for the diagnosis of PV are less stringent and lack a separate criterion for labeling *JAK2*-negative PV, while the BSH criteria are more stringent in terms of different hematocrit requirements and other additional criteria like splenomegaly and WBC counts. The frequency of *JAK2V617F* mutation in the present study in PV cases is less than in published studies [15–17]. Further research focusing on the identification of a possible genetic etiology for these *JAK2*-negative PV cases is warranted.

In this study group, the proportion of MPN patients with ET is less when compared with registry data from other Asian countries [9]. Comparison with previous studies showed that the mutation profile of ET cases in our study was different. *JAK2*-mutated ET cases were fewer, while *CALR*-mutated ET and triple-negative cases were higher (Table 3). *CALR*-mutated ET cases were younger, had significantly less hemoglobin, and greater platelet counts compared with the *JAK2*-mutated ET cases (Table 2). Triple-negative ET cases presented at a younger age with a lower hemoglobin and a higher platelet count than *JAK2*-mutated ET cases. This observation is in concordance with a previous report [18]. A lower overall median age of the ET patients in this study can be explained by the skewing of data due to a larger number of triple-negative ET cases who were younger at presentation.

The spectrum of mutations in the driver genes in patients with PMF was different in our study. In comparison to published data, though the frequency of *JAK2V617F*-mutated cases was comparable (~50%), detection of *JAK2* exon 12 mutations adds to the varied molecular spectrum of this group. *CALR*-mutated cases were higher while the frequencies of *MPL*-mutated PMF and triple-negative cases were comparable. *CALR*-mutated PMF cases were significantly younger and had significantly higher platelet counts than those with *JAK2*-mutated PMF (Table 2). However, there was no significant difference in hemoglobin and total leukocyte count as described in previous studies [19–21]. Among the *CALR*-mutated MPN, type 1 *CALR* mutation (52 bp deletion) was more common in PMF than in ET (75% vs. 44%) and type 2 *CALR* was more common in ET than PMF (52% vs. 17%) in our study. This is in concordance with previously published data [21,22].

In our study, 78.2% of the patients categorized as MPN-U carried a mutation in one of the three driver genes, with *JAK2V617F* being the most frequently

represented (65%) followed by *CALR* mutations (11.2%). This is comparable to a previous study on MPN-U [23]. A more complete molecular characterization of these cases would not only help in identifying the driver mutations, but also in understanding whether or not these cases represent only a prodromal or advanced phase of the classic *BCR-ABL1*-negative MPNs.

Previous study using the whole-exome sequencing approach on cases of triple-negative ET and PMF has identified noncanonical mutations in *JAK2* and *MPL* genes [24]. A possible hereditary thrombocytosis, owing to the predominant younger age at presentation (median age 18 years for triple-negative ET) in this group cannot be excluded.

Our study provides a large data set from India on the mutation spectrum and demographics of MPN. Future directions of this study are on the role of next generation sequencing in cases with features of MPNs where no mutation was identified in any of the driver genes (triple negative) in establishing clonality or excluding reactive conditions.

#### Authors' contributions

MM, UK, VM, PB: conceptualization; MM, UK, NR, AKA, AV, PB: data curation; UK, SL, AJD, AK, FNA, AA, AS, BG, VM: clinical data accrual; MM, PB, VM: funding acquisition; MM, PB, MTM: laboratory analysis; MM, UK, PB: original draft; VM, PB: approved the final version of the manuscript.

#### Ethical approval

The study was approved by the institutional research board (IRB Min No: 11800 dated January 30, 2019). All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research and ethics committee. The study and the laboratory tests have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

#### Declaration of Competing Interest

The authors declare that they have no competing interest.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.hemonc.2021.03.002>.

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