

Analysis of JAK2, TET2 and MYD88 gene mutations in BCR-ABL–negative myeloproliferative neoplasms (MPNs) and myelodysplastic/myeloproliferative neoplasms

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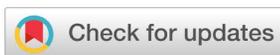
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ABSTRACT

Introduction: BCR-ABL-negative myeloproliferative neoplasms (MPNs)—including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF)—together with myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), are clinically heterogeneous chronic myeloid disorders. These entities are predominantly driven by somatic mutations in Janus kinase 2 (JAK2), calreticulin (CALR) and myeloproliferative leukemia (MPL) genes. Although JAK2 exon 12 and MYD88 mutations are reported less frequently, they may still contribute to disease pathogenesis and progression. This study aimed to investigate the mutational landscape of JAK2 (V617F and exon 12), TET2 and MYD88 in a Malaysian cohort of patients with BCR-ABL-negative MPNs or MDS/MPNs. **Methods:** A total of 54 patients with BCR-ABL-negative MPNs (n = 48) or MDS/MPNs (n = 6) were prospectively enrolled between 2015 and 2018. Genomic DNA isolated from peripheral blood was analyzed by polymerase chain reaction followed by Sanger sequencing. **Results:** The JAK2 V617F mutation was identified in 81.5 % of cases, including 90 % of PV, 84.6 % of ET and 80 % of PMF patients. Among the JAK2 exon 12 variants detected, D544N and c.1194+12G>A were consistently observed on repeat sequencing. These variants, previously reported by other investigators, were likewise present in our cohort, reinforcing their potential relevance to the molecular profile of MPNs. No pathogenic variants were found in TET2 exons 1 or 7. In addition, a known MYD88 intronic single-nucleotide polymorphism (rs4988457) was detected in three MPN patients (PV = 2, ET = 1), all of whom were co-mutated for JAK2 V617F and/or an exon 12 variant. **Conclusion:** This study confirms the central role of JAK2 V617F in BCR-ABL-negative MPNs and documents rare but potentially significant JAK2 exon 12 co-mutations. The presence of the MYD88 rs4988457 variant supports a possible inflammatory component in MPN pathogenesis. Together, these findings underscore the value of comprehensive mutational screening and provide further insight into the molecular complexity of MPNs, with implications for diagnosis, risk stratification and the development of targeted therapies.

Key words: Myeloproliferative neoplasms (MPNs), Myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), BCR-ABL–negative, JAK2 mutation, TET2 mutation, MYD88 mutation, Mutation prevalence

INTRODUCTION

Myeloproliferative neoplasms (MPNs) — comprising polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) — are rare clonal myeloid disorders originating in the bone marrow¹. These disorders are classified by the World Health Organization (WHO) as BCR-ABL-negative MPNs² and are frequently complicated by thromboembolic events, with potential progression to myelofibrosis or acute myeloid leukemia (AML)³. A related category, myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), exhibits overlapping features of both dysplasia and myeloid proliferation^{4,5}.

Somatic mutations constitute a cornerstone of the pathogenesis and classification of these disorders⁶. The JAK2 V617F mutation is detected in nearly all PV patients and in more than half of those with ET or PMF^{7–9}. Other driver mutations occur in the CALR and MPL genes, each converging on the JAK-STAT signaling pathway that regulates hematopoiesis^{10,11}. The less common JAK2 exon 12 mutation, highly specific for PV, facilitates the diagnosis of JAK2 V617F-negative patients^{12–14}. Additional mutations in ten-eleven translocation 2 (TET2), an epigenetic regulator acting downstream of JAK2¹⁵, are particularly observed in PMF^{16,17} and MDS/MPNs⁵. Myeloid differentiation primary response 88 (MYD88), an adaptor protein within the

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Toll-like receptor (TLR) signaling pathway, is classically associated with Waldenström's macroglobulinemia (WM)¹⁸, but recent studies have reported MYD88 variants in a subset of ET cases¹⁹, raising questions about their broader role in myeloid disorders.

Aberrant activation of both JAK-STAT and TLR signaling pathways has been implicated in abnormal hematopoiesis and immune dysfunction in these disorders^{20,21}.

In the present study, we characterize the mutational profiles of JAK2, TET2, and MYD88 in a cohort of BCR-ABL-negative MPN and MDS/MPN patients, thereby contributing to the existing knowledge of these disease conditions.

MATERIALS AND METHODS

Study design

This prospective cohort study enrolled patients treated at Hospital Universiti Sains Malaysia (USM) over a three-year period (2015–2018). Eligibility criteria comprised individuals with BCR-ABL-negative myeloproliferative neoplasms (MPNs)—PV, ET, and PMF—patients who subsequently progressed to secondary myelofibrosis, and those with MDS/MPNs. Diagnostic criteria followed the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues for myeloid neoplasms and acute leukaemia, 2008 edition and its 2016 revision.

Patient samples

The study enrolled patients diagnosed with either BCR-ABL-negative MPNs or MDS/MPNs who underwent JAK2 V617F mutation analysis at Hospital USM. Hematological parameters, including hemoglobin (Hb), platelet, red blood cell (RBC) and white blood cell (WBC) counts, as well as JAK2 V617F mutation status, were retrieved from the Hematology Laboratory Information System (LIS) of Hospital USM. Peripheral blood specimens from healthy controls (n = 12) were also collected. All procedures were conducted in accordance with the USM Human Research Ethics Committee guidelines (USM/JEPeM/20020079).

Genetic Analyses

Genomic DNA was isolated from peripheral whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). Mutational analysis was performed on JAK2 exon 12, TET2 exons 1 and 7, and MYD88 exons 3, 4, and 5 using Sanger sequencing. Primers

for JAK2 exon 12 were adopted from dos Santos et al. 2014²², whereas primers targeting TET2 exons 1 and 7 and MYD88 exons 3–5 were designed de novo with Primer3Plus (primer3plus.com) and validated with NCBI BLAST. Target fragments were amplified by polymerase chain reaction (PCR) with exTEN II PCR Master Mix (1st Base, Singapore) containing 100 ng of template DNA per reaction. Amplicons were verified on 1 % agarose gels and then sent to 1st Base (Singapore) for capillary Sanger sequencing. Primer sequences and PCR conditions are provided in Additional File—Table 1a.

Data and statistical analysis

Sequencing data analysis was conducted using Sequence Scanner software 2.0 (Thermo Fisher Scientific, USA). The identified sequence variations were cross-referenced with the single nucleotide polymorphism database (dbSNP) online tool to identify any existing polymorphisms. Statistical analysis was conducted utilizing the statistical software package GraphPad Prism version 9 (GraphPad Software, USA). Continuous variables are expressed as medians and ranges, whereas categorical variables are expressed as counts and percentages. The comparison of continuous variables was performed using an independent t test, whereas Fisher's exact test was applied for categorical variables. A p value <0.05 was considered to indicate statistical significance.

RESULTS

Demographics and Hematological Profiles

In this prospective study, a total of 54 patients were enrolled, comprising 48 with BCR-ABL-negative MPNs—20 with PV, 13 with ET, 11 with PMF, and 4 with post-PV MF—and 6 with MDS/MPNs. The median age was 63 years (range, 30–90 years), with 56.3 % male predominance. Hematologic parameters varied according to disease subtype. PV patients exhibited significantly elevated RBC counts (median, $6.9 \times 10^{12}/L$), WBC counts (median, $15.9 \times 10^9/L$), and platelet counts (median, $520.5 \times 10^9/L$). Patients with ET had a median platelet count of $789.0 \times 10^9/L$, whereas those with PMF presented with anemia (median hemoglobin, 10.0 g/dL), lower RBC counts (median, $4.1 \times 10^{12}/L$), and variable leukocytosis and thrombocytosis. Compared with BCR-ABL-negative MPNs, MDS/MPN cases demonstrated higher WBC and platelet counts (p = 0.0054), consistent with the hyperproliferative phenotype of this entity. The demographic and hematologic characteristics of the patients are summarized in Table 1a and Table 1b.

Table 1a: Demographics and hematological profiles of BCR-ABL–negative MPN patients (PV, ET, MF)

Patient’s characteristic and hematological profiles	MPN (n=48)	PV (n=20)	ET (n=13)	MF (n=15)	p-value (PV and ET)	p-value (PV and MF)	p-value (ET and MF)
Gender ^a							
Male	27 (56.3)	11 (55.0)	7 (53.8)	9 (60.0)	>0.9999	>0.9999	>0.9999
Female	21 (43.7)	9 (45.0)	6 (46.2)	6 (40.0)			
Age (years) ^b	63.0 (30.0-90.0)	65.0 (40.0-79.0)	55.0 (30.0-90.0)	61.0 (43.0-71.0)	0.1303	0.2119	0.4737
Age (years) ^a							
≤59	18 (37.5)	4 (20.0)	8 (61.5)	6 (40.0)	NA	NA	NA
60-69	19 (39.5)	10 (50.0)	2 (15.4)	7 (46.7)			
70-79	9 (18.8)	6 (30.0)	1 (7.7)	2 (13.3)			
≥80	2 (4.2)	0 (0.0)	2 (15.4)	0 (0.0)			
Complete blood counts ^b							
Hb (g/dL)	13.1 (5.4-23.4)	14.8 (8.8-23.4)	13.4 (10.2-14.8)	10.0 (5.4-16.5)	0.0184*	0.0001***	0.0138*
RBC count (x 10 ¹² /L)	5.3 (1.9-9.4)	6.9 (4.4-9.4)	5.0 (3.6-5.8)	4.1 (1.9-7.6)	<0.0001****	<0.0001****	0.4196
WBC count (x 10 ⁹ /L)	13.7 (4.0-82.8)	15.9 (7.1-31.3)	10.9 (7.2-36.5)	14.4 (4.0-82.8)	0.3185	0.0465*	0.0545
Platelet count (x 10 ⁹ /L)	516.0 (50.0-1752.0)	520.5 (195.0-1388.0)	789.0 (297.0-1611.0)	387.0 (50.0-1752.0)	0.0276*	0.1725	0.0068**

The cohort comprised 56.3% males and 43.7% females, with a median age of 63 years. Group comparisons revealed significant differences in Hb levels (across all MPN groups), RBC count (PV vs. ET and PV vs. MF), WBC count (PV vs. MF), and platelet count (PV vs. ET and ET vs. MF). ^an (Percentage), ^bMedian (range), an independent t-test was used to evaluate the p-value for continuous variables and Fisher’s exact test for categorical variables. NA: Not applicable

Mutation Profiling

JAK2 V617F Mutation

JAK2 V617F was identified in 44 patients (81.5%), including 18 with PV (90.0%), 11 with ET (84.6%), 12 with PMF (80.0%), and three with MDS/MPN (50.0%). In PV, mutation-positive individuals exhibited significantly higher platelet counts than mutation-negative counterparts (median 589.0 vs. 207.5 × 10⁹/L; p = 0.0359; Table 2). Among ET patients, those harboring JAK2 V617F were significantly older than wild-type cases (median 59.0 vs. 30.5 years; p = 0.0469; Table 2). In contrast, hematologic indices did not differ between JAK2-positive and JAK2-negative MDS/MPN patients, a finding that may reflect the limited cohort size (n = 6; Table 2).

JAK2 Exon 12 Mutation

JAK2 exon 12 mutations, D544N and c.1194+12G>A, were detected in six patients (11.1%): five with PV

and one with ET. A compound mutation involving both D544N and c.1194+12G>A was identified in one PV patient (PV3) (Additional File S2), whereas an isolated D544N mutation was observed in another PV patient (PV5) (Additional File S3); the latter finding was confirmed on repeat sequencing. The c.1194+12G>A mutation alone was detected in four patients (three with PV and one with ET) (Additional Files S5, S6, S7, and S9); this result was likewise confirmed in a second sequencing run. The allelic and predicted amino-acid sequences of the wild-type gene and the identified JAK2 exon 12/intron 12 variants are presented in Table 3. Neither D544N nor c.1194+12G>A was detected in reverse sequencing of normal controls (Additional Files S13 and S14). All cases positive for JAK2 exon 12 mutations also carried the JAK2 V617F mutation (Figure 2).

Table 1b: Demographic and hematological profiles of PMF, Post-PV MF, and MDS/MPN patients

Patient's characteristic and hematological profiles	PMF (n=11)	Post-PV MF (n=4)	p-value (PMF and post-PV MF)	MDS/MPN (n=6)	p-value (MDS/MPN and MPN)
Gender ^a					
Male	6 (54.5)	3 (75.0)	0.6044	3 (50.0)	>0.9999
Female	5 (45.5)	1 (25.0)		3 (50.0)	
Age (years) ^b	62.0 (43.0-71.0)	61.0 (55.0-68.0)	0.7009	62.0 (54.0-70.0)	0.7772
Age (years) ^a					
≤59	5 (45.5)	1 (25.0)	NA	3 (50.0)	NA
60-69	4 (36.4)	3 (75.0)		2 (33.3)	
70-79	2 (18.1)	0 (0.0)		1 (16.7)	
≥80	0 (0.0)	0 (0.0)		0 (0.0)	
Complete blood counts ^b					
Hb (g/dL)	9.1 (5.4-14.3)	11.8 (5.4-14.0)	0.9754	11.4 (5.4-14.3)	0.1509
RBC count (x 10 ¹² /L)	4.1 (1.9-6.9)	4.8 (3.9-7.6)	0.1944	4.4 (1.1-6.8)	0.0694
WBC count (x 10 ⁹ /L)	12.1 (4.0-82.8)	26.9 (9.0-55.6)	0.7971	18.2 (3.2-318.7)	0.0054**
Platelet count (x 10 ⁹ /L)	367.0 (50.0-717.0)	500.0 (385.0-1752.0)	0.0549	699.5 (241.0-1197.0)	0.9838

The cohort included 11 PMF patients (54.5% male), 4 post-PV MF patients (75% male), and 6 MDS/MPN patients (equally distributed between genders), with an age range of 61–62 years. A significant difference in WBC count was noted between MDS/MPN and MPN groups. ^an (percentage), ^bMedian (range). An independent t-test was used to evaluate the p-value for continuous variables and Fisher's exact test for categorical variables. NA: Not applicable

TET2 Mutation

No mutations were detected in exons 1 or 7 of TET2 in our study cohort.

MYD88 Mutation

We detected an intronic SNP, rs4988457 (1944C>G), in three MPN patients (PV = two patients; ET = one patient) (Figure 1A, 1B, 1C), all co-mutated with JAK2 V617F and/or exon 12. No MYD88 variants were found in PMF or MDS/MPNs. Correlation study with hematological parameters observed no significant differences between MYD88-positive and MYD88-negative patients within the PV and ET groups (Table 4).

DISCUSSION

This study evaluates the mutation profile of JAK2 V617F, JAK2 exon 12, TET2 and MYD88 among MPN and MDS/MPN patients.

JAK2 V617F mutation

The high prevalence of JAK2 V617F in our cohort (81.5%) is consistent with its well-established diagnostic value in BCR-ABL-negative MPNs according to WHO criteria^{23,24}. A separate Malaysian series documented the mutation in 73.6% of patients with MPN²⁵. Relative to neighbouring populations, the frequency was lower in Thailand (32.9%)²⁶, comparable in Singapore (77.5%)²⁷, and ranged from 56% to 67% in Chinese cohorts²⁸⁻³⁰. The markedly elevated platelet counts observed in PV cases harbouring JAK2 V617F imply a higher thrombotic risk, in line with previous evidence of JAK2-driven platelet activation³¹⁻³³. Among patients with ET, mutation-positive individuals were significantly older, supporting an age-related accumulation of the mutation as reported in earlier studies^{34,35}. Although earlier publications demonstrated significant differences in haematological indices between MDS/MPN patients stratified by JAK2 status³⁶, the present work detected no such differences, probably because

Table 2: Comparison of demographics, hematological and mutation profiles in JAK2 V617F-positive and JAK2 V617F-negative BCR-ABL-negative MPN patients

Patient's characteristic hematological and mutation profiles	JAK2 V617F-positive				JAK2 V617F-negative				P- value (PV)	P- value (ET)	P- value (MF)	P- value (MDS/ MPN)
	PV (n=18)	ET (n=11)	MF (n=12)	MDS/ MPN (n=3)	PV (n=2)	ET (n=2)	MF (n=3)	MDS/ MPN (n=3)				
Gender ^a												
Male	9 (50.0)	7 (63.3)	6 (50.0)	1 (33.3)	2 (100.0)	0 (0.0)	3 (100.0)	2 (66.7)	0.4789	0.1293	0.5147	>0.9999
Female	9 (50.0)	4 (36.4)	6 (50.0)	2 (66.7)	0 (0.0)	2 (100.0)	0 (0.0)	1 (33.3)				
Age (years) ^b	65.5 (40.0- 79.0)	59.0 (37.0- 90.0)	61.5 (43.0- 71.0)	67.0 (54.0- 70.0)	59.5 (58.0- 61.0)	30.5 (30.0- 31.0)	56.0 (47.0- 63.0)	57.0 (55.0- 68.0)	0.5165	0.0469*	0.3184	0.5951
Age (years) ^a												
≤59	3 (16.7)	6 (54.5)	5 (41.7)	1 (33.3)	1 (50.0)	2 (100.0)	1 (33.3)	2 (66.7)	NA	NA	NA	NA
60-69	9 (50.0)	2 (18.2)	5 (41.7)	1 (33.3)	1 (50.0)	0 (0.0)	2 (66.7)	1 (33.3)				
70-79	6 (33.3)	1 (9.1)	2 (16.6)	1 (33.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)				
≥80	0 (0.0)	2 (18.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)				
Complete blood counts ^b												
Hb (g/dL)	14.7 (8.8-23.4)	13.4 (10.2- 14.8)	10.4 (5.4-16.5)	12.7 (10.0- 13.8)	19.4 (17.6- 21.2)	11.9 (10.6- 13.1)	8.8 (8.4-11.1)	7.7 (5.4-14.3)	0.1260	0.5225	0.6012	0.3541
RBC count (x 10 ¹² /L)	6.9 (4.4-9.4)	5.0 (3.6-5.8)	4.3 (1.9-7.6)	5.0 (4.5-6.8)	6.6 (6.2-7.0)	4.2 (4.1-4.4)	4.1 (2.5-4.2)	3.1 (1.1-4.3)	0.4535	0.2537	0.3320	0.0934
WBC count (x 10 ⁹ /L)	15.9 (8.2-31.3)	12.4 (7.2-36.5)	26.9 (7.8-82.8)	23.4 (13.0- 49.9)	12.6 (7.1-18.2)	7.5 (7.4-7.7)	8.5 (4.0-14.4)	10.5 (3.2- 318.7)	0.7492	0.2785	0.1387	0.4765
Platelet count (x 10 ⁹ /L)	589.0 (363.0- 1388.0)	867.0 (297.0- 1611.0)	446.0 (80.0- 1752.0)	723.0 (367.0- 1197.0)	207.5 (195.0- 220.0)	487.0 (472.0- 502.0)	231.0 (50.0- 271.0)	680.0 (241.0- 719.0)	0.0359*	0.1160	0.1960	0.4915
Gene mutations ^a												
JAK2 exon 12	8 (44.4)	3 (27.3)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (33.3)	0 (0.0)	0.4737	>0.9999	0.2000	>0.9999

Table 2: Comparison of demographics, hematological and mutation profiles in JAK2 V617F-positive and JAK2 V617F-negative BCR-ABL-negative MPN patients (continued)

Patient's characteristic hematological and mutation profiles	JAK2 V617F-positive				JAK2 V617F-negative				p- value (PV)	p- value (ET)	p- value (MF)	p- value (MDS/ MPN)
	PV (n=18)	ET (n=11)	MF (n=12)	MDS/ MPN (n=3)	PV (n=2)	ET (n=2)	MF (n=3)	MDS/ MPN (n=3)				
<i>TET2</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	>0.9999	>0.9999	>0.9999	>0.9999
<i>MyD88</i>	2 (11.1)	1 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	>0.9999	>0.9999	>0.9999	>0.9999

Comparison of demographics, hematological parameters, and mutation profiles between JAK2 V617F-positive and JAK2 V617F-negative patients diagnosed with BCR-ABL-negative myelo-proliferative neoplasms (MPNs) and MDS/MPNs, including subgroups of polycythemia vera (PV), essential thrombocythemia (ET), myelofibrosis (MF), and MDS/MPN overlap syndromes. Statistically significant differences were observed in age among ET patients and in platelet counts among PV patients.^an (percentage), ^b Median (range). An independent *t*-test was used to evaluate the *p*-value for continuous variables and Fisher's exact test for categorical variables. NA: Not applicable

of the small sample size (n = 6). Overall, the robust association between JAK2 V617F and BCR-ABL-negative MPNs underscores its diagnostic and prognostic importance, although its precise relationship with clinical phenotype and thrombosis remains incompletely defined^{17,37–39}.

JAK2 exon 12 mutation

The analysis revealed that six patients (five with PV and one with ET) harbored a JAK2 exon 12 mutation. Worldwide, JAK2 exon 12 mutations account for approximately 2–5 % of PV cases, occur almost exclusively in JAK2-V617F-negative disease, and are exceedingly rare in ET/PMF and MDS/MPN^{40,41}.

Among the identified variants, D544N and c.1194+12G>A were consistently detected in repeat sequencing runs, suggesting that these variants are highly reliable. Previous literature has documented more than ten distinct sequence variations within the JAK2 exon 12 region, the majority of which cluster between codons 536 and 544⁴², which is a recognized mutation hotspot^{43–45}. Mutations occurring in this region may disrupt the JH2 pseudokinase domain, analogous to V617F, thereby resulting in constitutive activation of JAK-STAT signaling^{46,47}. Notably, alterations involving the E543–D544

sequence have been reported in multiple studies^{48,49}, and the specific D544N variant has been detected in the Chinese population²⁸. The c.1194+12G>A variant, located 12 nucleotides downstream of exon 12 within intron 12, lies in close proximity to a previously described variant, c.1194+6T>C, identified in the same genomic region²². Interestingly, all JAK2 exon 12-positive cases also harbored the JAK2 V617F mutation. This co-occurrence is atypical, as exon 12 mutations are generally considered mutually exclusive with V617F in classical MPNs⁵⁰. Nonetheless, familial and complex PV cases with such co-mutations have been reported^{48,51}. A study from Qatar (Western Asia) likewise reported a high frequency of JAK2 exon 12 mutations in PV and ET that co-occurred with JAK2 V617F⁵², suggesting possible geographical variation. The co-detection of JAK2 V617F and exon 12 variants may indicate subclonal heterogeneity arising from clonal mosaicism and disease evolution, although such events are rare⁵³. Because our analysis relied on Sanger sequencing—which is less sensitive than next-generation sequencing (NGS) and susceptible to artefacts—accurate quantification of low-level variant allele frequencies (VAF < 15–20 %) was not feasible. Accordingly, definitive conclusions cannot be drawn, and this observation warrants further investigation.

Table 3: Allele and predicted amino acid sequences of wild-type and detected *JAK2* exon 12 and intron 12 variants

	Exon 12																Intron 12			
Wild-type	ATG	AAC	CAA	ATG	GTG	TTT	CAC	AAA	ATC	AGA	AAT	GAA	GAT	TTG	ATA	TTT	GTA	AGT	CAT	TAG
	M	N	Q	M	V	F	H	K	I	R	N	E	D	L	I	F	-	-	-	-
V1	ATA	AAC	CAA	ATG	GTG	TTT	CAC	AAA	ATC	AGA	AAT	GAA	GAT	TTG	ATA	TTT	GTA	AGT	CAT	TAG
M532I	I	N	Q	M	V	F	H	K	I	R	N	E	D	L	I	F	-	-	-	-
V2	ATG	AAC	CAA	AGG	GTG	TTT	CAC	AAA	ATC	AGA	AAT	GAA	GAT	TTG	ATA	TTT	GTA	AGT	CAT	TAG
M535R	M	N	Q	R	V	F	H	K	I	R	N	E	D	L	I	F	-	-	-	-
V3	ATG	AAC	CAA	ATG	GGG	TTT	CAC	AAA	ATC	AGA	AAT	GAA	GAT	TTG	ATA	TTT	GTA	AGT	CAT	TAG
V536G	M	N	Q	M	G	F	H	K	I	R	N	E	D	L	I	F	-	-	-	-
V4	ATG	AAC	CAA	ATG	GTG	TTT	CAC	AAA	ATC	AAA	AAT	GAA	GAT	TTG	ATA	TTT	GTA	AGT	CAT	TAG
R541K	M	N	Q	M	V	F	H	K	I	K	N	E	D	L	I	F	-	-	-	-
V5	ATG	AAC	CAA	ATG	GTG	TTT	CAC	AAA	ATC	AGA	AAT	GAA	AAT	TTG	ATA	TTT	GTA	AGT	CAT	TAG
D544N	M	N	Q	M	V	F	H	K	I	R	N	E	N	L	I	F	-	-	-	-
V6	ATG	AAC	CAA	ATG	GTG	TTT	CAC	AAA	ATG	AGA	AAT	GAA	GAT	TTG	ATA	TTT	GTA	AGT	CAT	TAA
c.1194+12G>A	M	N	Q	M	V	F	H	K	I	R	N	E	D	L	I	F	-	-	-	-
V7	ATG	AAC	CAA	AGG	TGT	TTC	ACA	AAA	TCA	GAA	ATG	AAG	ATT	TGA	TAT	TTG	TAA	GTC	ATT	AGA
c.1157delT	M	N	Q	R	C	F	T	K	S	E	M	K	I	-	-	-	-	-	-	-
V8	ATG	AAC	CAA	ATG	GGT	TTC	ACA	AAA	TCA	GAA	ATG	AAG	ATT	TGA	TAT	TTG	TAA	GTC	ATT	AGA
c.1160delT	M	N	Q	M	G	F	T	K	S	E	M	K	I	-	-	-	-	-	-	-

Normal capital letters indicated the nucleotides and bold capital letters indicated amino acids. Letters in red represented changes in the nucleotides and amino acids. V1: variant 1 (M532I); V2: variant 2 (M535R); V3: variant 3 (V536G); V4: variant 4 (R541K); V5: variant 5 (D544N); V6: variant 6 (c.1194+12G>A); V7: (c.1157delT); V8: (c.1160delT).

Table 4: Comparison of demographics, hematological and mutation profiles in MYD88-positive and MYD88-negative BCR-ABL-negative MPN patients

Patient's demographics, hematological and mutation profiles	MYD88-positive		MYD88-negative		P-value (PV)	P-value (ET)
	PV (n=2)	ET (n=1)	PV (n=18)	ET (n=12)		
Gender^a						
Male	1 (50.0)	0 (0.0)	10 (55.6)	7 (58.3)	>0.9999	0.4615
Female	1 (50.0)	1 (100.0)	8 (44.4)	5 (41.7)		
Age (years)^b	69.0 (66.0-72.0)	42.0	64.5 (40.0-79.0)	57.0 (30.0-90.0)	0.4006	0.5000
Age (years)^a						
≤59	0 (0.0)	1 (100.0)	4 (22.2)	7 (58.3)	NA	NA
60-69	1 (50.0)	0 (0.0)	9 (50.0)	2 (16.7)		
70-79	1 (50.0)	0 (0.0)	5 (27.8)	1 (8.3)		
≥80	0 (0.0)	0 (0.0)	0 (0.0)	2 (16.7)		
Complete blood counts^b						
Hb (g/dL)	17.5 (16.4-18.6)	13.4	14.7 (8.8-23.4)	13.3 (10.2-14.8)	0.4394	0.6588
RBC count (x 10 ¹² /L)	6.6 (6.2-7.1)	4.6	6.9 (4.4-9.4)	5.0 (3.6-5.8)	0.7531	0.8508
WBC count (x 10 ⁹ /L)	14.5 (10.4-18.5)	7.2	15.9 (7.1-31.3)	11.7 (7.2-36.5)	0.7586	0.4428
Platelet count (x 10 ⁹ /L)	759.0 (475.0-1043.0)	867.0	520.5 (195.0-1388.0)	779.0 (297.0-867.0)	0.5238	0.9136
Gene mutations^a						
JAK2 V617F	2 (100.0)	1 (100.0)	16 (88.9)	10 (83.3)	>0.9999	>0.9999
JAK2 exon 12	2 (100.0)	0 (0.0)	7 (38.9)	3 (25.0)	0.1895	>0.9999
TET2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	>0.9999	>0.9999

No significant differences were observed between MYD88-positive and MYD88-negative patients within the PV and ET groups: ^a n (percent), ^b Median (range). An independent t-test was used to evaluate the p-value for continuous variables and Fisher's exact test for categorical variables. NA: Not applicable

TET2 mutation

A recent meta-analysis reported TET2 mutation prevalences of 20.8 % in Asia, 17.4 % in North America, 13.0 % in Europe, and 7.0 % in Australia⁵⁴. Although TET2 is a common epigenetic modifier in MPNs, we did not detect mutations in exons 1 or 7 within our cohort. This observation aligns with prior studies demonstrating that pathogenic variants cluster predominantly in exons 3 and 11⁵⁵. Exon 1 contains promoter sequences that regulate gene expression, whereas exon 7 resides within a conserved domain. The absence of variants in these

exons may therefore reflect either a low mutation frequency or epigenetic silencing mechanisms that are not detectable by conventional sequencing⁵⁶⁻⁵⁸. Moreover, previous reports suggest that TET2 mutations alone are insufficient to initiate the MPN phenotype, although they may facilitate genomic instability and disease progression³. The apparent absence of TET2 variants in our series may thus stem from the restricted exon coverage rather than their true biological absence. Comprehensive sequencing of the entire coding region will therefore be required to delineate the full TET2 mutational landscape.

MYD88 mutation

We identified three patients with MPNs—two with PV and one with ET—harboring the rare rs4988457 (c.1944C>G) MYD88 variant. Consistent with previous reports, MYD88 mutations are exceedingly uncommon in BCR-ABL–negative MPNs. In large sequencing series, including a cohort of 303 individuals, no MYD88 alterations were detected⁵⁹, with the exception of a single ET case complicated by Waldenström’s macroglobulinemia (WM), in which a MYD88 variant was observed¹⁹. Accordingly, our study is among the first to evaluate MYD88 mutations in BCR-ABL-negative MPNs and in MDS/MPN overlap syndromes. Although the pathobiological significance of rs4988457 remains undefined, MYD88 aberrations have been implicated in multiple other hematologic malignancies, including T-cell lymphomas⁶⁰, activated B-cell–type diffuse large B-cell lymphoma (ABC-DLBCL)⁶¹, chronic lymphocytic leukemia (CLL)⁶², and AML⁶³.

CONCLUSIONS

This report provides insights into the mutational landscape of BCR-ABL-negative MPNs and myelodysplastic/myeloproliferative neoplasms (MDS/MPNs) in a Malaysian cohort, with an emphasis on JAK2, TET2, and MYD88 alterations. The overall mutational profile is illustrated in Figure 2. The high prevalence of the JAK2 V617F mutation confirms its pivotal diagnostic role across PV, ET, PMF, and MDS/MPNs. JAK2 exon 12 variants, specifically D544N and c.1194+12G>A, previously reported in other studies, were likewise identified in our cohort. Finally, identification of the intronic MYD88 variant rs4988457 in three MPN cases suggests a potential inflammatory contribution to MPN pathogenesis and warrants further investigation.

CONSENT

The study protocol was approved by the Human Research Ethics Committee of University Sains Malaysia (USM) (Reference number: USM/JEPeM/20020079). Informed consent was obtained from the MPN and MDS/MPN patients prior to sample collection.

ABBREVIATIONS

ABC-DLBCL: Activated B-cell diffuse large B-cell lymphoma; **AML:** Acute myeloid leukemia; **BCR-ABL:** Breakpoint cluster region–Abelson proto-oncogene; **BM:** Bone marrow; **CALR:** Calreticulin; **c.1194+12G>A:** Intronic substitution in

the JAK2 gene, where guanine (G) is replaced by adenine (A) at 12 nucleotides downstream of coding position 1194 (intron 12); **CLL:** Chronic lymphocytic leukemia; **D544N:** Aspartic acid (D) to Asparagine (N) substitution at codon 544 of the JAK2 gene; **ET:** Essential thrombocythemia; **Hb:** Hemoglobin; **JAK2:** Janus kinase 2; **MF:** Myelofibrosis; **MPL:** Myeloproliferative leukemia virus oncogene; **MPN:** Myeloproliferative neoplasm; **MDS/MPN:** Myelodysplastic/myeloproliferative neoplasm; **MYD88:** Myeloid differentiation primary response 88; **NGS:** Next-generation sequencing; **PCR:** Polymerase chain reaction; **PMF:** Primary myelofibrosis; **Post-PV MF:** Post-polycythemia vera myelofibrosis; **PV:** Polycythemia vera; **RBC:** Red blood cell; **SNP:** Single nucleotide polymorphism; **TLR:** Toll-like receptor; **VAF:** Variant allele frequency; **V617F:** Valine (V) at amino acid position 617 is replaced by Phenylalanine (F); **WBC:** White blood cell; **WHO:** World Health Organization; **WM:** Waldenström’s macroglobulinemia

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AUTHOR’S CONTRIBUTIONS

M.R., M.A.I and M.F.J conceptualized the study and designed the methodology, Y.C.C performed the data collection, M.J.S.A and Y.C.C performed the data analysis and wrote the main manuscript text, M.J.S.A wrote the final manuscript revision, M.J.S.A and M.A.I checked for intellectual content and editing. M.R., R.H., and M.F.J supervised the project. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare that they have not used generative AI (a type of artificial intelligence technology that can produce various types of content including text, imagery, audio and synthetic data. Examples include ChatGPT, NovelAI, Jasper AI, Rytr AI, DALL-E, etc) and AI-assisted technologies in the writing process before submission.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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