CME/SAM

Comparison of the Mutational Profiles of Primary Myelofibrosis, Polycythemia Vera, and Essential Thrombocytosis

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ABSTRACT

Objectives: *To compare the mutational profiles of patients with primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocytosis (ET).*

Methods: *Next-generation sequencing results of 75 cases of PMF, 33 cases of PV, and 27 cases of ET were compared.*

Results: *Mutation rates of* ASXL1 *and* SRSF2 *were significantly higher in PMF than in PV or ET.* ASXL1 *mutations appeared to be more frequently associated with risk of transformation to acute myeloid leukemia than* JAK2 *or* TET2 *mutations. The most common mutation-cytogenetic combinations in myeloproliferative neoplasm (MPN) were mutations of* JAK2 *or* ASXL1 *with del(20q) and were more common in patients with PMF and PV than in patients with ET. Differences were also found between patients with PMF and PV.*

Conclusions: *PMF, PV, and ET show different mutational profiles, which may be helpful in resolving the differential diagnosis between MPNs. Due to the relatively small number of cases and variable testing over time, larger controlled studies are necessary to confirm the findings.*

Upon completion of this activity you will be able to:
- discuss the application of gene mutations in the differential diagnosis of primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocytosis (ET).
- discuss the significance of gene mutations in predicting prognosis of patients with PMF, PV, or ET.

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Primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocytosis (ET) are common Philadelphia chromosome–negative chronic myeloproliferative neoplasms (MPNs), characterized by cytoses, splenomegaly, and hypercellular bone marrows with proliferation of myeloid, erythroid, and/or megakaryocytic lineages. In early stage disease, PMF usually presents with leukocytosis, PV with increased hemoglobin level, and ET with thrombocytosis. However, it is a challenge to distinguish between ET and prefibrotic/early myelofibrosis, as well as initial cases of PV from ET or even PMF.[1-3] The differential diagnosis at the intermediate stage is easier but could still be a pathologic challenge. The relevance of differentiating between these entities lies beyond an academic exercise given the different natural histories and treatment approaches for each. Survival is longest in ET (median survival of 20

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years), intermediate in PV (14 years), and shortest in PMF (6 years).[1,4] The goal of therapy in PV and ET is aimed more at supportive care and prevention of thrombocytosis than curing disease. In contrast, for patients with genetically and clinically high-risk PMF, stem cell transplant is the treatment of choice.[1,4] Different prognostic systems are also used for these three identities.[1,4] Therefore, there is clinical utility in identifying unique nonmorphologic features that may help to distinguish between these three entities. As these diseases progress, they all may potentially develop diffuse bone marrow fibrosis and present with cytopenia(s). At the terminal fibrotic stage, marrow morphologies can be very similar between advanced PMF and post-ET/PV myelofibrosis, making them hard to distinguish and posing a real and common diagnostic challenge to the pathologist. However, the prognosis and the treatment may be similar between these entities at this stage.[5]

With rapid progress in the sequencing technology due to next-generation sequencing (NGS), somatic mutations in growing numbers of genes, with pathogenetic and prognostic significance, have been identified in myeloid neoplasms. Key MPN-specific driver mutations have been well characterized—namely, *JAK2*, *CALR*, and *MPL*—and these are usually mutually exclusive.[6] *JAK2* is the most frequent mutation in Philadelphia chromosome–negative MPN, with frequencies of approximately 95% to 98% in PV, 50% to 60% in ET, and 55% to 65% in PMF.[4] *CALR* and *MPL* mutations are typically absent in patients with PV with rare exception. *CALR* mutations are present in approximately 20% to 25% of ET or PMF cases, while *MPL* mutations are present in 3% to 4% of ET cases and 6% to 7% of PMF cases.[4]

It has been previously shown that an MPN can be initiated from a single hematopoietic stem cell expressing the canonical *JAK2* V617 mutation.[7] Furthermore, *JAK2* inhibitors have shown promising activity in controlling constitutional symptoms and splenomegaly in PMF[8] and PV but have not been shown to be disease modifying. Patients with *CALR* mutations have been reported to have lower risk of thrombosis in ET and better overall survival in PMF compared with *JAK2*-mutated patients.[9] Among patients with ET, those with *MPL* mutations show significantly inferior overall survival.

Of note, approximately 10% to 15% of patients with PMF or ET do not express any of the three (*JAK2*, *CALR*, *MPL*) mutations. However, with the widespread research and clinical application of NGS, mutations in additional genes and other nondriver mutations have been identified in MPN, and their clinical and prognostic significance is under intensive studies.[10] Given the growing body of genomic data, it has been suggested that MPNs may better be categorized based on their mutational bases rather than morphologic differences with regard to clinical outcomes.[11]

In this study, we analyzed the mutational profiles and cytogenetic abnormalities of 135 patients with PMF, PV, or ET with the aim of uncovering molecular and/or cytogenetic profiles that may facilitate the distinction between and understanding of these three entities.

Materials and Methods

Selection of Patients

This study was approved by the institutional review board (IRB) of Moffitt Cancer Center. NGS for myeloid neoplasms by commercial or in-house laboratories with College of American Pathologists/Clinical Laboratory Improvement Amendments (CAP/CLIA) certification was started at our institute in May 2013. All the patients with NGS data from May 2013 to July 2015 were retrieved per IRB protocols. The clinical ambulatory reports, pathology reports, and pathology slides were reviewed by two board-certified hematopathologists to confirm or revise the diagnosis as appropriate. Diagnoses were rendered following the World Health Organization 2008 classification for hematopoietic malignancies. Patients with a confirmed diagnosis of PMF, PV, or ET and NGS mutational panels were included in this study. A total of 135 patients were identified, including 75 with PMF, 33 with PV, and 27 with ET. Most cases (>95%) carried a clear and consistent diagnosis at the time of NGS. A few cases, especially those with PMF, did not have a clear diagnosis at the time of NGS but were given consistent diagnosis of PMF in later repeat bone marrow biopsies. One case of PV was incorrectly diagnosed as ET. The cytogenetic results of all these patients, including karyotyping and fluorescence in situ hybridization (FISH) study results (mostly FISH for the myelodysplastic syndrome [MDS] panel, including del(5q), del(7q), del(17p), del(20), and trisomy 8), were also retrieved from the electronic database and analyzed.

NGS Analysis

NGS was performed at a CLIA-certified commercial laboratory between May 2013 and October 2014, first by a five-gene panel (*ASXL1*, *RUNX1*, *EZH2*, *ETV6*, *TP53*) and then a 21-gene panel (*SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*, *TET2*, *IDH1*, *IDH2*, *DNMT3A*, *EZH2*, *ASXL1*, *SETBP1*, *TP53*, *PHF6*, *RUNX1*, *ETV6*, *CBL*, *NRAS*, *KIT*, *JAK2*, *MPL*, *NPM1*). Starting from October 2014, NGS was performed in-house using a 31-gene panel (*ABL1*, *ASXL1*, *CBL*, *CEBPA*, *CSF3R*, *CUX1*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KMT2A*, *KRAS*, *MPL*, *MYD88*, *NPM1*, *NRAS*, *PHF6*, *RUNX1*, *SETBP1*, *SF3B1*, *SH2B3*, *SRSF2*, *TET2*, *TP53*, *U2AF1*, *WT1*, *ZRSR2*). All patients with suspected MPNs, including PMF, PV, and ET, were

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submitted to molecular tests to a panel that was available at the time of submission. Of the 135 patients included in this study, 65 were tested by the 31-gene panel, 37 by the 21-gene panel, and two by the five-gene panel. Thirty patients with PMF and one patient with PV were only tested for single genes, mostly *JAK2* or *MPL*, before the outside 21-gene panel and in-house 31-gene panels were started. *CALR* was not included in any the panels and therefore was tested for very few patients. Since any patients with a diagnosis of PMF, PV, or ET and any mutation information were included in our study, it happened that none of the genes was tested for every patient, and different genes have a different number of tested patients. Since very few patients were tested for *CALR*, it is not used to derive any information in this study. Most of the tests were performed on the peripheral blood (120, including 63 patients with PMF, 31 with PV, and 26 with ET). The remaining patients, most with PMF, were tested on the bone marrow, with five being hemodilute and 10 being adequate.

Both the outside laboratory and in-house testing are CAP/CLIA-certified laboratories. The in-house NGS platform was validated against the results of the same outside laboratory for quality control at the validation stage and confirmed that the results were comparable. In addition, the analysis of just the 65 patients who were all tested by the in-house platform yielded similar overall mutation rates to the mutations rates when all 135 patients were analyzed (data not shown). This also supports the assumption that the results from in-house and outside laboratories are comparable. However, a further larger cohort study using the same testing platforms is necessary to confirm the results of this study. In-house targeted NGS was performed using the Illumina MiSeq or NexSeq500 instruments (Illumina, San Diego, CA). DNA was isolated with a QiaAmp DNA extraction kit and the QiaCube robot (Qiagen, Germantown, MD) and quantitated by Nanodrop spectrophotometry (Agilent, Santa Clara, CA). Library construction was performed with 250 ng genomic DNA using the using amplicon-based capture for 31 genes. FastQ files were analyzed by the Clinical Genomicist Workspace (PierianDX, St Louis, MO) to identify clinically significant variants. FastQ files were aligned using Novalign (Selangor, Malaysia), and various bioinformatic tools, including Samtools, Varscan, and Freebayes in PierianDX pipeline, were used to make variant calls. The outside laboratory isolated DNA from bone marrow aspirates or peripheral blood, and then five genes or coding regions (117 exons) of 21 genes were amplified by polymerase chain reaction and interrogated by NGS technology.

All tests reported variants with an allele frequency of 5% or more. For patients with NGS testing on more than one instance, a gene mutated in any of the tests was considered mutated for that patient. A gene that was mutated in multiple samples from the same patient was counted only once when enumerating the number of the patients who were positive for the mutations of that gene. Known benign single nucleotide polymorphisms (SNPs) were excluded. Variants with more than 1% minor allele frequency in the Single Nucleotide Polymorphism database or the National Heart, Lung, and Blood Institute Exome Sequencing Project database were also considered nonsomatic mutations and excluded. When determining the percentage of mutated patients for a gene, the number of patients positive for mutations was divided by the number of patients tested for that gene to compensate for the different panels used for different patients.

Table 1
Demographics and Mutation Rates

Characteristic	PMF (n = 75)	PV (n = 33)	ET (n = 27)
Median age, y	72	68	63
Male/female, No. (ratio)	44/31 (1.42)	18/15 (1.2)	9/18 (0.5)
No. (%) of patients with ≥1 mutation	61/75 (81.33)	32/33 (96.97)	25/27 (92.59)

ET, essential thrombocytosis; PMF, primary myelofibrosis; PV, polycythemia vera.

Statistical Analysis

Two-way Fisher exact test was used to calculate the *P* values when comparing the percentages of positive patients.

Results

Patient Characteristics

We searched our electronic database for patients with a diagnosis of PMF, PV, or ET and with NGS results. In total, 135 patients were identified **Table 1**. The median age was 69 years for all patients. There were 64 female patients and 71 male patients. These included 75 patients with PMF (median age, 72 years; male-to-female ratio of 1.42; four cases transformed into acute myeloid leukemia [AML]), 33 patients with PV (median age, 68 years; male-to-female ratio of 1.2; two cases transformed into AML), and 27 patients with ET (median age, 63 years; male-to-female ratio of 0.5; one case transformed into AML). The mean years from diagnosis and the fibrosis stages of the patients are shown in **Table 2**.

Overall Gene Mutation Rates in Patients With MPN

A total of 32 genes were tested: *ABL1*, *ASXL1*, *CALR*, *CBL*, *CEBPA*, *CSF3R*, *CUX1*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KMT2A*, *KRAS*, *MPL*, *MYD88*, *NPM1*, *NRAS*, *PHF6*, *RUNX1*, *SETBP1*, *SF3B1*, *SH2B3*, *SRSF2*, *TET2*, *TP53*, *U2AF1*, *WT1*, and *ZRSR2*. Of

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Table 2
Mean Years From Diagnosis and Fibrosis Stages

Disease	Mean Years From Diagnosis	Prefibrotic, No./Total No. (%)	Fibrotic, No./Total No. (%)	AML Transformation, No./Total No. (%)
PMF	5.08	30/75 (40.44)	41/75 (30.30)	4/75 (5.33)
PV	11.2	21/33 (63.64)	10/33 (30.30)	2/33 (6.06)
ET	7.7	22/27 (81.48)	4/27 (14.81)	1/27 (3.70)

AML, acute myeloid leukemia; ET, essential thrombocytosis; PMF, primary myelofibrosis; PV, polycythemia vera.

Table 3
Mutational Rates of Genes in All Patients

Gene	No. of Patients (PMF/PV/ET)	No. of Patients Tested	No. (%) of Patients Mutated
JAK2	72/33/27	132	87 (65.91)
CALR	17/0/2	19	6 (31.58)
ASXL1	45/32/27	104	22 (21.15)
TET2	44/32/27	103	20 (19.42)
SRSF2	43/32/27	102	8 (7.84)
MPL	52/32/27	111	8 (7.21)
SF3B1	43/32/27	102	7 (6.86)
DNMT3A	44/32/27	103	7 (6.80)
KMT2A	29/22/14	65	4 (6.15)
CBL	43/32/27	102	6 (5.88)
IDH2	44/32/27	103	5 (4.85)
FLT3	30/22/14	66	3 (4.55)
U2AF1	43/32/27	102	4 (3.92)
EZH2	44/32/27	103	4 (3.88)
CUX1	29/22/14	65	2 (3.08)
ETV6	44/32/27	103	3 (2.91)
PHF6	44/32/27	103	3 (2.91)
IDH1	44/32/27	103	2 (1.94)
TP53	44/32/27	103	2 (1.94)
CSF3R	29/22/14	65	1 (1.54)
KRAS	29/22/14	65	1 (1.54)
SH2B3	29/22/14	65	1 (1.54)
NRAS	43/32/27	102	1 (0.98)
ZRSR2	43/32/27	102	1 (0.98)
KIT	44/32/27	103	1 (0.97)
ABL1	29/22/14	65	0 (0.00)
CEBPA	29/22/14	65	0 (0.00)
MYD88	29/22/14	65	0 (0.00)
NPM1	44/32/27	103	0 (0.00)
RUNX1	44/32/27	103	0 (0.00)
SETBP1	43/32/27	102	0 (0.00)
WT1	29/22/14	65	0 (0.00)

ET, essential thrombocytosis; PMF, primary myelofibrosis; PV, polycythemia vera.

these genes, 25 were found to be mutated in at least one of the patients. The genes that were never mutated in our patients with MPN included *ABL1*, *CEBPA*, *MYD88*, *NPM1*, *RUNX1*, *SETBP1*, and *WT1*.

The percentages of patient with at least one gene mutated were 81.33% for PMF, 96.97% for PV, and 92.59% for ET (Table 1). The mutation rate of patients with PV, mostly *JAK2* mutations, was statistically higher than that for patients with PMF ($P = .0347$).

Table 3 delineates the overall gene mutation rates of all patients with MPN in our study. As expected, *JAK2* had the highest mutation rate (65.91%), followed by *CALR* (31.58%), *ASXL1* (21.15%), *TET2* (19.42%), *SRSF2* (7.84%), *MPL* (7.21%), *SF3B1* (6.86%), *DNMT3A* (6.80%), *KMT2A* (MLL) (6.15%), and *CBL* (5.88%). The remaining genes showed a mutation rate of less than 5%. Since much fewer patients were tested for *CALR* (19 patients), this gene was not included in the following analysis or comparison to preclude bias. To address the potential influence of selection bias, we also analyzed just the 65 patients who were tested for all 31 genes by the in-house platform. The overall mutation frequencies of all the genes

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Table 4
Difference in Mutation Rate of *JAK2*, *ASXL1*, and *SRSF2* Among Patients With PMF, PV, and ET[a]

	PMF		PV		ET	
Gene	No./Total No. (%)	*P* Value	No./Total No. (%)	*P* Value	No./Total No. (%)	*P* Value
JAK2	39/72 (54.17)	.001	**31/33 (93.94)**[b]	NA	17/27 (62.96)	.0038
ASXL1	**18/45 (40.00)**[c]	NA	2/32 (6.25)	.0012	2/27 (7.41)	.0027
SRSF2	**8/43 (18.60)**[c]	NA	0/32 (0.00)	.0178	0/27 (0.00)	.0196

ET, essential thrombocytosis; PMF, primary myelofibrosis; PV, polycythemia vera.
[a]*P* values listed are the *P* values when comparing the highest percentage (in bold) of mutations of the gene with other percentages. Since not all patients with PMF were tested for these genes, 95% confidence intervals are calculated for patients with PMF as follows: *JAK2* (48.4-59.5), *ASXL1* (33.7-46.9), and *SRSF2* (13.8-24.3).
[b]Percentages that are highest in PV.
[c]Percentages that are highest in PMF.

Table 5
Genes With Different Mutation Rates in PMF, PV, and ET but Not Statistically Significant ($P > .05$)

Gene	PMF, No./Total No. (%)	PV, No./Total No. (%)	ET, No./Total No. (%)
TET2	**12/44 (27.27)**[a]	6/32 (18.75)	2/27 (7.41)
MPL	**7/52 (13.64)**[a]	0/32 (0.00)	1/27 (3.70)
U2AF1	**4/43 (9.30)**[a]	0/32 (0.00)	0/27 (0.00)
CUX1	**2/29 (6.90)**[a]	0/32 (0.00)	0/14 (0.00)
IDH2	**4/44 (9.09)**[a]	0/32 (0.00)	1/27 (3.70)
KMT2A	1/29 (3.45)	**3/22 (13.64)**[b]	0/14 (0.00)
TP53	0/44 (0.00)	**2/32 (6.25)**[b]	0/27 (0.00)
ETV6	0/44 (0.00)	0/32 (0.00)	**3/27 (11.11)**[c]
CSF3R	0/29 (0.00)	0/32 (0.00)	**1/14 (7.14)**[c]

ET, essential thrombocytosis; PMF, primary myelofibrosis; PV, polycythemia vera.
[a]Percentages that are highest in PMF.
[b]Percentages that are highest in PV.
[c]Percentages that are highest in ET.

are similar to those when all 135 patients were all analyzed (data not shown).

Different Mutation Profiles of PMF, PV, and ET

Table 4 shows genes with statistically significantly different mutation rates in PMF, PV, and ET. *JAK2* showed a higher mutation rate in PV (93.94%) than in PMF (54.17%, $P = .001$) and ET (62.96%, $P = .0038$). *ASXL1* demonstrated a much higher mutation rate in PMF (40.0%) than in PV (6.25%) and ET (7.41%) ($P = .0012$ and $P = .0027$, respectively). *SRSF2* demonstrated a higher mutation rate in PMF (18.60%) than in PV and ET (both 0%) ($P = .0178$ and $P = .0196$, respectively). To address the potential influence of variable numbers of genes tested for different patients, we also analyzed the mutation rates of *JAK2*, *ASXL1*, and *SRSF2* just for the 65 patients who were tested for all 31 genes for PMF, PV, and ET. The mutation frequencies of these three genes in each category are similar to those in Table 4 when 135 patients were all analyzed (data not shown). These findings indicate the special roles *ASXL1* and *SRSF2* may play in PMF and potential diagnostic utility of mutations in these genes in resolving the differential diagnosis.

Several other genes appeared to have different mutation rates in these three entities but did not reach statistical significance, which could be due to inadequate sample numbers in this study **Table 5**. The mutation rate of *TET2* was highest in PMF (27.27%), intermediate in PV (18.75%), and lowest in ET (7.41%). Other genes that appear to have higher mutation rates in PMF compared with PV or ET include *MPL*, *U2AF1*, *CUX1*, and *IDH2*, in order of decreasing frequency. Mutation rates of *KMT2A* (*MLL*) and *TP53* appeared to be higher in PV (13.64% and 6.25%, respectively) than in PMF (3.45% and 0%, respectively) and ET (0% and 0%, respectively). On the other hand, mutations of *ETV6* and *CSF3R* were detected in ET (11.11% and 7.14%, respectively) but not in PMF or PV. Larger cohort studies are necessary to confirm and clarify these findings.

The remaining genes showed no obvious difference in mutational rates between these three entities (data not shown).

Patients With *ASXL1*, *JAK2*, and *TET2* Mutations and Transformation to AML

JAK2, *ASXL1*, and *TET2* were the most frequently mutated genes in our MPN patient cohort (*CALR* is not listed due to much fewer tested patients in this cohort). In this study, we analyzed the patients who were positive for these mutations to look for an association with transformation into AML **Table 6**. Among patients with PMF, 18 patients

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▌Table 6▌
***ASXL1*, *JAK2*, and *TET2* Mutations and the Rates of Transformation to AML**

	PMF		PV		ET	
Gene	Mutated, No.	AML, No. (%)	Mutated, No.	AML, No. (%)	Mutated, No.	AML, No. (%)
ASXL1	18	3 (16.67)	2	0 (0.00)	2	0 (0.00)
JAK2	39	1 (2.56)	31	2 (6.45)	17	1 (5.88)
TET2	12	0 (0.00)	6	0 (0.00)	2	0 (0.00)

AML, acute myeloid leukemia; ET, essential thrombocytosis; PMF, primary myelofibrosis; PV, polycythemia vera.
[a]*ASXL1*, *JAK2*, and *TET2* are not mutually exclusive. Comutations of these three genes in this study in patients with at least one of these three mutations are 28.3% in PMF, 25.81% in PV, and 5% in ET. The numbers of patients were too small for statistical analysis. Of the patients who had disease that transformed into AML, only one patient showed a comutation (*ASXL1* and *JAK2*).

were positive for *ASXL1* mutations, 39 patients were positive for *JAK2* mutations, and 12 patients were positive for *TET2* mutations. Four cases of PMF transformed into AML. Of the 18 cases of PMF that were positive for *ASXL1* mutations, three (16.67%) transformed into AML. Of the 39 cases of PMF that were positive for *JAK2* mutations, one (2.56%) transformed into AML. Of the 12 cases of PMF that were positive for *TET2* mutations, none transformed into AML. Therefore, patients with *ASXL1*-mutated PMF appeared to have a high rate of transformation into AML compared with those with *JAK2* or *TET2* mutations. In particular, PMF with *ASXL1* mutations might have a higher chance to transform into AML than PMF with *JAK2* or *TET2* mutations ($P = .08$ and $P = .25$, respectively). There were too few patients with PV or ET who had *ASXL1* mutations for meaningful statistical comparison in the ET and PV cohorts (two patients each).

Please note that *ASXL1*, *JAK2*, and *TET2* are not mutually exclusive. Comutations of these three genes in this study in the patients with at least one of these three mutations were 28.3% in PMF, 25.81% in PV, and 5% in ET (data not shown). The numbers of patients were too small for statistical analysis. Of the seven cases that transformed into AML, only one patient showed a comutation (*ASXL1* and *JAK2)*.

Cytogenetic Abnormalities in Patients With MPN

Of the 135 patients included in this study, 99 patients had karyotyping results with or without FISH studies for the MDS panel, which includes del(5), del(7), del(20), del(17), and trisomy 8. Three patients only had FISH study results and had no karyotyping results. Therefore, 102 (3 + 99) is used as the total number of tested patients for the five cytogenetic abnormalities in the FISH panel, while 99 is used as the total number of tested patients for the remaining cytogenetic abnormalities that are not included in the FISH panel. **▌Table 7▌** shows the most common cytogenetic abnormalities in the patients with MPN in our cohort. FISH for the MDS panel (del(5), del(7), del(20), del(17), and trisomy 8) was the panel that was most commonly performed. Of all patients with MPN, deletion 20q12 had the highest rate of occurrence (16.67%), followed by deletion 13q14.3 (8.08%) and trisomy 8 (5.88%). When subdivided into subcategories, patients with PMF tended to show more cytogenetic abnormalities than patients with PV and ET **▌Table 8▌**.

▌Table 7▌
Cytogenetic Abnormalities, All Patients[a]

Cytogenetic Abnormality	No. of Patients Tested	No. (%) of Patients Positive
del(20)	102	17 (16.67)
del(13)	99	8 (8.08)
tri(8)	102	6 (5.88)
tri(9)	99	4 (4.04)
del(5)	102	3 (2.94)
del(11)	99	2 (2.02)
del(7)	102	2 (1.96)
add(1)	99	1 (1.01)
add(11)	99	1 (1.01)
add(13)	99	1 (1.01)
add(14)	99	1 (1.01)
add(17)	99	1 (1.01)
add(18)	99	1 (1.01)
add(2)	99	1 (1.01)
add(6)	99	1 (1.01)
del(12)	99	1 (1.01)
del(14)	99	1 (1.01)
del(16)	99	1 (1.01)
del(18)	99	1 (1.01)
del(2)	99	1 (1.01)
del(21)	99	1 (1.01)
del(6)	99	1 (1.01)
der(1)t(1;5)	99	1 (1.01)
der(13;14)	99	1 (1.01)
der(2)t(2;17)	99	1 (1.01)
der(4)t(4;6)	99	1 (1.01)
der(8)t(1;8)	99	1 (1.01)
der(9)t(1;9)	99	1 (1.01)
der(9)t(5;9)	99	1 (1.01)
iso(17q)	99	1 (1.01)
q(13.3)	99	1 (1.01)
t(11;17)	99	1 (1.01)
t(12;17)	99	1 (1.01)
t(3;12)	99	1 (1.01)
t(3;4)	99	1 (1.01)
del(17)	102	1 (0.98)

[a]Ninety-nine patients had karyotyping results with or without fluorescence in situ hybridization (FISH) studies for the myelodysplastic syndrome panel, which includes del(5), del(7), del(20), del(17), and trisomy 8. Three patients only had FISH study results and had no karyotyping results. Therefore, 102 (3 + 99) is used as the total number of tested patients for the five cytogenetic abnormalities in the FISH panel.

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Table 8
Most Common Cytogenetic Abnormalities by Disease Subgroup

Cytogenetic Abnormality	PMF, No./Total No. (%)	PV, No./Total No. (%)	ET, No./Total No. (%)
del(20)	14/65 (21.54)	3/20 (15.00)	0/17 (0.00)
del(13)	7/62 (11.29)	0/20 (0.00)	1/17 (5.88)
tri(8)	6/65 (9.23)	0/20 (0.00)	0/17 (0.00)

ET, essential thrombocytosis; PMF, primary myelofibrosis; PV, polycythemia vera.

Table 9
Co-occurring Gene Mutations and Cytogenetic Abnormalities by Subgroup

Gene	Cytogenetic Abnormality	PMF, No./Total No. (%)	PV, No./Total No. (%)	ET, No./Total No. (%)
JAK2	del(20)	**12/61 (19.67)**[a]	3/20 (15.00)	0/17 (0.00)
ASXL1	del(20)	**7/37 (18.92)**[a]	0/20 (0.00)	0/17 (0.00)
ASXL1	tri(8)	**4/37 (10.81)**[a]	0/20 (0.00)	0/17 (0.00)
SRSF2	del(20)	**3/35 (8.57)**[a]	0/20 (0.00)	0/17 (0.00)
TP53	del(17)	0/36 (0.00)	**2/19 (10.53)**[b]	0/17 (0.00)
TP53	del(5)	0/36 (0.00)	**2/19 (10.53)**[b]	0/17 (0.00)
JAK2	del(17)	0/61 (0.00)	**2/20 (10.00)**[b]	0/17 (0.00)
JAK2	del(5)	0/61 (0.00)	**2/20 (10.00)**[b]	0/17 (0.00)
JAK2	tri(9)	1/61 (1.64)	**2/20 (10.00)**[b]	0/17 (0.00)

ET, essential thrombocytosis; PMF, primary myelofibrosis; PV, polycythemia vera.
[a]Percentages that are highest in PMF.
[b]Percentages that are highest in PV.

del(20) was seen in PMF and PV but not in ET. del(13) was seen in PMF and ET but not in PV. Finally, trisomy 8 was seen in PMF but not in PV and ET in this cohort. Due to the small number of cases in this study, statistical significance was not reached.

Co-occurrence of Gene Mutation and Cytogenetic Abnormalities in Patients With PMF, PV, or ET

In this study, we also analyzed the data to identify gene mutations and the cytogenetic abnormalities that occurred together in these MPNs. Mutation of *JAK2* and del(20q) occurred together most often (15.31%), followed by *ASXL1* and del(20q) (9.59%) and *ASXL1* and trisomy 8 (5.48%). High rates of co-occurrences of *JAK2* and *ASXL1* mutations with del(20q) are not surprising given that they were commonly mutated genes and a cytogenetic abnormality in our study.

In subgroup analysis **Table 9**, it is found that the combination of mutations and cytogenetic abnormalities occurred most commonly in PMF and PV compared with ET. JAK2 and del(20q) occurred in PMF (19.67%) and PV (15.0%) but not in ET. The combination of gene mutation and cytogenetic abnormality also shows different patterns between PMF and PV. The following combinations were found in PMF but not in PV: *ASXL1* mutation and del(20q), *ASXL1* mutation and trisomy 8, and *SRSF2* mutation and del(20q). On the other hand, the combination of *TP53* mutation and del(17), *TP53* mutation and del(5), *JAK2* mutation and del(17), and *JAK2* mutation and del(5) occurred in PV but not in PMF. Again, statistical significance was not reached, likely due to inadequate sample size.

Discussion

PMF, PV, and ET are three Philadelphia chromosome–negative chronic MPNs that can be difficult to distinguish based on morphologic grounds, particularly when in the prefibrotic and fibrotic phases. Despite the advances of the genomic era, limited information is available regarding differences in their genetic profiles/signatures. *JAK2*, *CALR*, and *MPL* are already well-recognized driver mutations in these entities. Somatic mutations in other genes, such as *TET2*, *DNMT3A*, *ASXL1*, *EZH2*, *IDH1/2*, *U2AF1*, *SF3B1*, *SRSF2*, *CBL*, *NF-E2*, *SH2B3 (LNK)*, *CHEK2*, *SOCS1*, *SOCS2*, *OCS3*, and *IKZF*, among others, have also been found in all stages of MPN.[12-18] These mutations are not MPN specific and have also been detected in many other myeloid disorders. They have also been found in patients harboring *JAK2*, *CALR*, or *MPL* mutations and therefore might cooperate to contribute to the pathogenesis of MPN by serving as secondary acquired mutations.[19] In this study, we analyzed NGS data and the cytogenetic abnormalities of 135 patients with PMF, PV, or ET to find out potential biomarkers that may further aid in distinguishing between these

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three entities. Since our institute is a tertiary institute, our patient population is somewhat different from the general patient population and tends to consist of more patients with refractory diseases.

In our study, *JAK2*, *ASXL1*, and *TET2* were the most frequently mutated genes in PMF, PV, or ET cases tested, while *RUNX1*, *SETBP1*, and *NPM1*, which are often mutated in other myeloid neoplasms such as MDS or AML, were not seen. Consistent with previous reports, *JAK2* showed the highest mutation rate in PV compared with PMF and ET. Overall, these three entities were found to have different mutation profiles. Mutation rates of *ASXL1* and *SRSF2* were higher in PMF than in PV and ET, and this difference was statistically significant. This not only suggests the potential role *ASXL1* and *SRSF2* play in PMF pathogenesis but also provides potential biomarkers that can aid in morphologically challenging cases. The presence of *ASXL1* and/or *SRSF2* makes the diagnosis of PMF more likely.

PMF tends to have a higher rate of transformation into AML than PV or ET. Only a few patients in our study (n = 7) had disease that transformed into AML. Four of our 75 cases of PMF transformed into AML, which is lower than the reported incidence of transformation for PMF (∼20%). This might be due to the limited sample size and short follow-up time of this study or because many patients came for transplant and therefore were not in the AML stage. Patients with PMF who had *ASXL1* mutations in our study appeared to have higher rate of transformation into AML than patients with PMF who had *JAK2* or *TET2* mutations. Other studies have also reported an association of *ASXL1* mutations with unfavorable survival in patients with PMF, independent of Dynamic International Prognostic Scoring System (DIPSS-plus) risk category.[4] One study reported increasing *ASXL1* mutation burden during transformation from PMF to AML.[20] Other genes that have been shown to have prognostic values in PMF include *SRSF2*, *EZH2*, *IDH1/IDH2*, and *U2AF1*.[21-23] *TP53*, *IDH2*, *SRSF2*, and *SH2B3* have been reported to be overrepresented in blast-phase MPN.[24,25] A recent study of 570 patients with PMF reported the longest survival in *CALR*+ *ASXL1*– patients (median 10.4 years), intermediate survival (5.8 years) in *CALR*+ *ASXL1*+ patients, and shortest survival (2.3 years) in *CALR*– *ASXL1*+ patients.[26]

In addition, *MPL*, *U2AF1*, and *CUX1* appeared to be mutated more frequently in PMF in this study than either PV or ET (Table 5); *KMT2A* and *TP53* appeared to have higher mutation rates in PV; and *ETV6* and *CSF3R* appeared to be more likely to be mutated in ET. These findings did not reach statistical significance, which may be due to the inadequate sample size in study subgroups or could simply represent insignificant findings. Further studies are required to clarify the significance of these differences. Of note, it has been shown in one study that *U2AF1* together with *JAK2* was found to have a high mutation burden in all stages of a patient with PMF that eventually transformed into AML, suggesting its role as founding clone.[20]

In this study, we also analyzed the cytogenetic abnormalities of our patients with MPN. Consistent with previous studies, del(20q), del(13q), and trisomy 8 were the most common cytogenetic abnormalities in PMF, PV, or ET. The subcategories of MPN revealed slightly different cytogenetic patterns. PMF tended to show more cytogenetic abnormalities than PV and ET. del(20q) appeared to be less common in ET, del(13) less common in PV, and trisomy 8 less common in PV and ET. When all patients were analyzed together, the most common combinations of mutation and cytogenetic abnormalities were *JAK2* or *ASXL1* with del(20). When divided into subcategories, the combinations of mutation and cytogenetic abnormalities were found to occur mostly in PMF and PV but not in ET, perhaps indicating much simpler pathogenesis in patients with ET. Differences in the mutation cytogenetic combinations were also found between PMF and PV: the combination of *ASXL1* mutation and del(20), *ASXL1* mutation and trisomy 8, and *SRSF2* mutation and del(20q) appeared to be more common in PMF, while the combination of *TP53* mutation and del(17), *TP53* mutation and del(5), *JAK2* mutation and del(17), and *JAK2* mutation and del(5) appeared to be more common in PV. These differences need to be confirmed by larger cohort studies. The combination of *ASXL1* mutation and del(20) has been reported to be associated with a shorter 2-year survival in patients with myelodysplasia.[27]

In conclusion, the differences in mutation and cytogenetic profiles of PMF, PV, and ET show some promise, albeit limited, in the differential diagnosis of these entities. One weakness of this study is the heterogeneity of specimens, and different patients were tested for different gene panels with different platforms, which might cause biased results. Larger controlled cohorts by the same gene panel by the same testing platform, at the same disease stage, and with homogeneous specimens are necessary to further confirm the results of this study. Nonetheless, our results show that genetic profiling may have a role in triaging and consequently guiding the different treatments of these three entities. For example, prevention of thrombosis is the major goal for patients with PV and ET. On the other hand, allogeneic stem cell transplant is the only potentially curative treatment in PMF and is therefore recommended in either DIPSS-plus high or molecular high PMF (absence of type/type 1–like *CALR* mutation and presence of *ASXL1* or related high-risk mutation).[26] Response to imetelstat appears to be positively influenced by the presence of *JAK2*, *SF3B1*, or *U2AF1* mutations and the absence of *ASXL1* mutations.[28] Even the order in which the mutations, such as *JAK2* and *TET2*, were acquired was found to influence the clinical features and the response to targeted therapy.[29] Further studies

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are necessary to more accurately define the impacts of different mutated genes, combined mutations, allele burdens, and the clonal evolution on the clinical presentation and prognosis of these clinically unique but morphologically and genetically overlapping Philadelphia chromosome–negative chronic myeloproliferative disorders.

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