Association of *JAK2* Mutation Status and Cytogenetic Abnormalities in Myeloproliferative Neoplasms and Myelodysplastic/Myeloproliferative Neoplasms

Jennifer Dunlap, MD,[1] Katalin Kelemen, MD, PhD,[1] Nicky Leeborg, MD,[1] Rita Braziel, MD,[1] Susan Olson, PhD,[2] Richard Press, MD, PhD,[1] James Huang, MD,[3] Ken Gatter, JD, MD,[1] Marc Loriaux, MD, PhD,[1] and Guang Fan, MD, PhD[1]

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Abstract

Myeloproliferative neoplasms and myelodysplastic/myeloproliferative neoplasms are heterogeneous disorders. JAK2 *mutation testing and karyotyping are routinely used for diagnosis but have not been incorporated into risk stratification in Philadelphia chromosome–negative myeloproliferative neoplasms. This study correlated cytogenetic abnormalities with disease stage and* JAK2 *status. A total of 179 cases were analyzed for the* JAK2 *mutation. Among them, cytogenetic data were available for 97 cases—45 of 106* JAK2+ *and 52 of 73* JAK2–. *The* JAK2+ *group showed a higher frequency of cytogenetic anomalies than the* JAK2– *group (23/45 [51%] vs 14/52 [27%]). Chromosome 9, chromosome 7, and 20q– were recurrent abnormalities in the* JAK2+ *group, whereas 13q– and trisomy 21 were common in the* JAK2– *group. In the* JAK2+ *group, chromosome 7 and complex cytogenetic abnormalities were associated with excess blasts/blastic transformation (*P *< .05), whereas no cases with 20q– underwent blastic transformation. Our results suggest that incorporation of* JAK2 *mutation testing and karyotyping allows for monitoring of disease progression with prognostic and therapeutic implications.*

Myeloproliferative neoplasms (MPNs) are clonal disorders of hematopoietic stem cells characterized by proliferation of 1 or more myeloid lineages. In the revised 2008 World Health Organization (WHO) classification of MPNs, the mutational status of *JAK2*, a tyrosine kinase involved in intracellular signaling, is an essential part of the diagnostic algorithm.[1] The *JAK2*^{V617F} point mutation has been reported in more than 90% of patients with polycythemia vera (PV) and approximately half of patients with primary myelofibrosis (PMF) and essential thrombocythemia (ET).[2-5] *JAK2* exon 12 mutations account for a significant number of *JAK2*^{V617F}– PV cases.[6,7]

Also included in the WHO classification of myeloid malignancies are myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), which show characteristics of myelodysplastic syndrome (MDS) and MPN. MDS/MPN demonstrates a significant albeit lower frequency of *JAK2* mutations (<10%).[2,8,9] The clinical course of MPN and MDS/MPN varies markedly within the same diagnostic subtype, and the existing prognostic system is mainly based on peripheral blood parameters and constitutional symptoms. Cytogenetic and molecular aberrations have historically not been a predominant part of the risk stratification system. In particular, the study of cytogenetic abnormalities to stratify disease prognosis in most types of MPN and MDS/MPN is still in its early stages compared with acute myeloid leukemias (AMLs). In contrast, in PMF, investigators have proposed that karyotypic abnormalities provide relevant prognostic information and have suggested a new international prognostic scoring system incorporating cytogenetic risk categorization.[10-12] In comparison, cytogenetic studies in ET and PV have not yielded consistent prognostic information, even though some common recurrent cytogenetic abnormalities have been identified,

including 20q–, trisomy 9, 13q–, trisomy 8, and others.[13-17] Often, the number of patients in each study is too small for investigators to be certain about a particular genetic abnormality. It is still controversial whether cytogenetic abnormalities should be included in a risk stratification scheme for ET or PV. Furthermore, the prevalence and pattern of cytogenetic abnormalities for *JAK2*+ vs *JAK2*– MPN and MDS/MPN have not been established.

The aim of this study was to determine whether cytogenetic data and *JAK2* mutation status provide prognostic information by analyzing cytogenetic abnormalities with *JAK2* mutational status data and correlating with the pathologic stage in cases of MPN and MDS/MPN, such as myelofibrosis or blastic transformation, from our single institution experience.

Materials and Methods

Cases

The study was approved by the Oregon Health & Science University (OHSU, Portland) Institutional Review Board. The OHSU surgical pathology files and clinical history database were searched for the period from 2005 to 2010 to identify cases of *JAK2*+ and *JAK2*– MPNs and MDS/MPNs (with and without cytogenetic data). The diagnoses and subclassifications were made according to WHO diagnostic criteria by using morphologic findings and clinical data. A total of 106 *JAK2*+ and 73 *JAK2*– cases were identified. The subclassification of these *JAK2*+ and *JAK2*– cases is provided in **Table 1**.

Table 1
Summary of Cases Tested for the *JAK2* Mutation

Classification/Subclassification/ Disease Stage	No. of *JAK2*+ Cases	No. of *JAK2*– Cases
MPN		
ET		
ET	24	19
Post-ET MF	3	3
Post-ET MDS	1	0
ET, blast phase	0	1
PV		
PV	26	2
Post-PV MF	7	0
Post-PV CMML	1	0
PV, blast phase	6	0
PMF		
PMF	20	18
PMF with excess blasts	1	1
PMF, blast phase	2	0
MPN		
MPN, unclassifiable	6	1
MPN, blast phase	2	0
Other		
SM-AHNMD	2	5
MDS/MPN		
aCML		
aCML, blast phase	0	2
CMML		
CMML	2	11
CMML-2	0	4
Post-CMML MF	0	1
CMML, blast phase	2	2
Other		
MDS/MPN, unclassifiable	0	2
RARS-T	1	1
Total	106	73

aCML, atypical chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; ET, essential thrombocythemia; MDS, myelodysplastic syndrome; MDS/MPN, myelodysplastic/myeloproliferative neoplasms; MF, myelofibrosis; MPN, myeloproliferative neoplasm; PMF, primary myelofibrosis; PV, polycythemia vera; RARS-T, refractory anemia with ringed sideroblasts associated with marked thrombocytosis; SM-AHNMD, systemic mastocytosis with associated clonal hematologic non–mast cell lineage disease.

JAK2 Mutational Analysis

Testing for the $JAK2^{V617F}$ mutation was performed by allele-specific polymerase chain reaction (PCR) on DNA prepared from bone marrow or peripheral blood specimens according to the method of Jones et al.[4] This method uses 1 primer pair to specifically amplify the normal (wild-type) sequence (229-base-pair product) and another primer pair to specifically amplify the mutant sequence (279 base pairs). The PCR products were sized by capillary electrophoresis (QIAxcel analyzer, Qiagen, Valencia, CA), which was routinely able to detect the V617 mutant allele down to a dilution limit (into wild-type DNA) of 0.5%. A 0.5% low mutant-allele burden control was included in each batch run of genotypes, and, if the mutant allele-specific PCR signal of any unknown sample exceeded that of the low-level (0.5%) control, it was interpreted as "positive" for $JAK2^{V617F}$.

Cytogenetics

Standard cytogenetic karyotype analysis was performed at the OHSU cytogenetics laboratory at the time of bone marrow biopsy or aspiration on 45 *JAK2*+ cases and 52 *JAK2*– cases. The bone marrow specimen was cultured for 24 to 48 hours in complete RPMI 1640 medium (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (Irvine Scientific, Santa Ana, CA). Cells were harvested and slides prepared according to standard laboratory protocol. Slides were treated with 10% trypsin (Invitrogen) for 40 to 55 seconds followed by Wright stain (Sigma, St Louis, MO) for 2 minutes, 30 seconds. These GTW-banded preparations were analyzed on a Nikon Eclipse E800 microscope (Nikon Instruments, Melville, NY) with Applied Imaging CytoVysion software (Genetix, San Jose, CA).[18] When possible, at least 20 metaphase cells were examined for each case.

Statistics

We assessed the univariate relationship between each of the predictor variables (hemoglobin level, WBC count, platelet count, age, normal cytogenetics, 20q deletion, chromosome 7 abnormalities, chromosome 9 abnormalities, monosomal karyotype, and complex cytogenetic abnormalities [>3 chromosome abnormalities]) with the outcome variable

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(blastic transformation/excess blasts) for *JAK2*+ cases by using univariate logistic regression. Then we fit a multivariate logistic regression to the data. All *P* values were 2-tailed, and statistical significance was set at the level of *P* less than .05. Analysis was performed using SAS software, version 9.2 (SAS Institute, Cary, NC) **Table 2**.

Results

Comparison of Diagnostic Subtype Distribution Between *JAK2*+ and *JAK2*– MPNs and MDS/MPNs

A search of OHSU pathology files for the period from January 2005 to March 2010 yielded a total of 179 cases with a diagnosis of MPN or MDS/MPN: 106 with a *JAK2* mutation and 73 without a *JAK2* mutation. The cases are listed in Table 1 according to diagnostic categories. As expected, the distribution of MPN and MDS/MPN subtypes varied between the *JAK2*+ and *JAK2*– groups. For the *JAK2*+ group, PV was the most common diagnosis (37.7%), followed by ET (26.4%), PMF (21.7%), MPN unclassifiable (5.7%), chronic myelomonocytic leukemia (CMML; 3.8%), systemic mastocytosis with associated clonal hematologic non–mast cell lineage disease (SM-AHNMD; 1.9%), and refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T; 0.9%). For the *JAK2*– group, ET was the most common subtype (23/73 [32%]) followed by PMF (19/73 [26%]), CMML (18/73 [25%]), SM-AHNMD (5/73 [7%]), atypical chronic myeloid leukemia (2/73 [3%]), MPN unclassifiable (2/73 [3%]), and RARS-T (1/73 [1%]). As we expected, PV was significantly more frequent in the *JAK2*+ group and CMML was significantly more frequent in the *JAK2*– subset.

Cytogenetic Findings in *JAK2*+ MPN and MDS/MPN

Overall, 45 of 106 *JAK2*+ cases had cytogenetic data available for review. Diagnostic subclassification and corresponding cytogenetic findings are given in **Table 3**. Cytogenetic abnormalities were identified in 23 (51%) of 45 cases, with frequencies of 25% for CMML (1/4), 40% for ET (2/5), 53% for PV (8/15), and 60% for PMF (9/15). The occurrence of cytogenetic abnormalities in cases with blastic transformation or excess blasts was significantly higher (12/14 [86%]) than in cases without (11/31 [35%]; $P < .05$). Recurrent cytogenetic abnormalities in the *JAK2*+ group included 20q–, chromosome 7 abnormalities (7q– and –7), chromosome 9 abnormalities [trisomy 9 and t(1;9)], chromosome 17 abnormalities (17q– and isochromosome 17q), and chromosome 5 abnormalities (5q– and monosomy 5). The relationship between cytogenetic abnormalities and outcome (blastic transformation/excess blasts) was analyzed using univariate logistic regression and multivariate analysis and is shown in Table 2.

In our series, 20q– as the sole cytogenetic abnormality was seen in 4 *JAK2*+ cases with abnormal cytogenetics, including 1 post–PV MF case, 2 PMF cases, and 1 MPN case; none of these cases underwent blastic transformation, suggesting that 20q– is associated with a better prognosis, although this did not reach statistical significance perhaps owing to the small sample.

Chromosome 9 abnormalities such as trisomy 9 and t(1;9) were seen in 5 cases. Of the 5 cases, 4 (80%) underwent transformation to AML, including 2 post-PV cases with blastic transformation, 1 PMF case with blastic transformation, and 1 CMML case with blastic transformation. For the case of CMML with blastic transformation, the original clone demonstrated trisomy 9 at diagnosis that evolved into a tetraploid chromosome 9 at the time of transformation. For the case of PV with blast phase, the original clone demonstrated trisomy 9, and an additional new deletion of 7q– was seen at the time of blastic transformation.

Three cases demonstrated a monosomal karyotype defined as the presence of 2 distinct autosomal monosomies or a single autosomal monosomy in the presence of other structural abnormalities. The diagnoses included 1 case of

Table 2
Clinical Data and Cytogenetic Abnormalities in 45 *JAK2*+ Cases With and Without Blastic Transformation*

Characteristic	All Cases	Cases With Transformation/ Excess Blasts (n = 15)	Cases With No Transformation (n = 30)	*P*†
Age (y)	62.7 (mean)	63.9 ± 8.5	62.1 ± 13.9	.6486
M/F	25/20	8/7	17/13	1.0000
Hemoglobin level, g/dL (g/L)	12.9 ± 4.14 (129 ± 41)	13 ± 6.0 (130 ± 60)	12.8 ± 3.0 (128 ± 30)	.8815
WBC count, /μL (× 10^9/L)	14,740 ± 12,000 (14.74 ± 12)	14,300 ± 16,700 (14.3 ± 16.7)	14,900 ± 9,100 (14.9 ± 9.1)	.8726
Platelet count, × 10^3/μL (× 10^9/L)	342.9 ± 364.4 (342.9 ± 364.4)	280 ± 228 (280 ± 228)	374.3 ± 416.4 (374.3 ± 416.4)	.4206
20q–	4	0	4	.2847
Chromosome 9 abnormalities	5	4	1	.036
Chromosome 7 abnormalities‡	7	5	2	.0322
Monosomal karyotype	3	3	0	.0202
Complex cytogenetic abnormalities‡	4	4	0	.0048

* Data are given as number of cases or mean ± SD unless otherwise indicated.
† By univariate analysis.
‡ $P < .05$ in multivariate analysis.

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PV, 1 case of MDS/MPN, and 1 case of MPN, all of which underwent blastic transformation.

By using univariate analysis, we found that chromosome 9 abnormalities and monosomal karyotype were associated with blastic transformation; however, this association was not significant by multivariate analysis, although the number of cases is small.

Chromosome 7 abnormalities (7q– or monosomy 7) were also commonly seen (7/45 cases). In 3 cases, there was a sole chromosome 7 abnormality, 3 cases had an abnormal chromosome 7 with complex cytogenetic abnormalities (>3 chromosome abnormalities), and 1 case had trisomy 9. A total 5 of 7 cases had blastic transformation, including 4 cases of PV, blast phase, and 1 case of MDS/MPN, blast phase. The association of chromosome 7 abnormalities with blastic transformation was statistically significant ($P < .05$) by univariate and multivariate analyses, indicating this cytogenetic abnormality portends a worse prognosis in *JAK2*+ cases.

Table 3
***JAK2*+ Cases With Cytogenetic Data**

Case No./Classification	Disease Stage	Cytogenetics
ET		
1	ET	Normal
2	Post–ET MF	Normal
3	Post–ET MF	Normal
4	Post–ET MF	7q–
5	Post–ET MF RAEB-2	t(12;17)
PV		
6	PV	Normal
7	PV	Normal
8	PV	Gain 1q
9	Post–PV MF	Normal
10	Post–PV MF	Normal
11	Post–PV MF	Normal
12	Post–PV MF	20q–
13	Post–PV MF	t(1;9) extra derivative 9
14	Post–PV CMML	Normal
15	PV, blast phase	Normal
16	PV, blast phase	Trisomy 9
17	PV, blast phase	7q–
18	PV, blast phase	Trisomy 9 with addition of 7q– at blastic phase
19	PV, blast phase	Derivative chr 7 with 1q, abnormal chr 18, trisomy 21
20	PV, blast phase	Monosomy 5, monosomy 7, 12q–, 17q–
PMF		
21	PMF	Normal
22	PMF	Normal
23	PMF	Normal
24	PMF	Normal
25	PMF	Normal
26	PMF	Normal
27	PMF	Trisomy 8
28	PMF	20q–
29	PMF	20q–
30	PMF	t(2;12)
31	PMF	t(10;12)
32	PMF with excess blasts 2	Monosomy 7
33	PMF with excess blasts 2	Abnormal chr 1 and chr 6
34	PMF, blast phase	Isochromosome 17q
35	PMF, blast phase	t(1;9)
MPN		
36	MPN, unclassifiable	20q–
37	MPN, blast phase	5q–, trisomy 13, monosomy 16, isochromosome 17q
SM-AHNMD		
38	SM-AHNMD	Normal
39	SM-AHNMD	Normal
MDS/MPN		
40	RARS-T	Normal
41	MDS/MPN, blast phase	5q–, abnormal chr 6, monosomy 7, abnormal chr 12, Xq13
42	CMML	Normal
43	CMML	Normal
44	CMML, blast phase	Normal
45	CMML, blast phase	Trisomy 9 to tetraploid chr 9

chr, chromosome; CMML, chronic myelomonocytic leukemia; ET, essential thrombocythemia; MDS/MPN, myelodysplastic/myeloproliferative neoplasms; MF, myelofibrosis; MPN, myeloproliferative neoplasm; PMF, primary myelofibrosis; PV, polycythemia vera; RAEB, refractory anemia with excess blasts; RARS-T, refractory anemia with ringed sideroblasts associated with marked thrombocytosis; SM-AHNMD, systemic mastocytosis with associated clonal hematologic non–mast cell lineage disease.

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Other common cytogenetic abnormalities involved chromosomes 5 and 17. All were associated with complex cytogenetic abnormalities and related to excess blasts or blastic transformation ($P < .05$).

Cytogenetic Findings in *JAK2*– MPN and MDS/MPN

Among the *JAK2*– cases, 52 MPN, MDS/MPN, and MDS cases had cytogenetic data available for review **Table 4**. A total of 14 cases (27%) showed abnormal cytogenetics, with

Table 4
***JAK2*– Cases With Cytogenetic Data**

Case No./Classification	Disease Stage	Cytogenetics
ET		
1	ET	Normal
2	ET	Normal
3	ET	Normal
4	ET	Normal
5	ET	Normal
6	ET	Normal
7	ET	Normal
8	ET	Normal
9	Post–ET MF	Normal
10	Post–ET MF	Normal
11	Post–ET MF	Inversion 12
12	ET, blast phase	Trisomy 14, trisomy 19, trisomy 21
PMF		
13	PMF	Normal
14	PMF	Normal
15	PMF	Normal
16	PMF	Normal
17	PMF	Normal
18	PMF	Normal
19	PMF	Normal
20	PMF	Normal
21	PMF	Normal
22	PMF	Ring chr 6
23	PMF	t(8;9) *PCM1-JAK2*
24	PMF	13q–
25	PMF	13q–
26	PMF	20q–
27	PMF	Abnormal chr 5, chr 6, and chr 22, 7q–
28	PMF with excess blasts 2	13p–, 15q–, monosomy 2, monosomy 20
SM-AHNMD		
29	SM-AHNMD	Normal
30	SM-AHNMD	Normal
31	SM-AHNMD	Abnormal chr 4
32	SM-AHNMD with excess blasts	Normal
33	SM-AHNMD, CMML	Normal
MDS/MPN		
34	MDS/MPN, unclassifiable	t(5;12)
35	aCML MF, blast phase	Normal
36	CMML	Normal
37	CMML	Normal
38	CMML	Normal
39	CMML	Normal
40	CMML	Normal
41	CMML	Normal
42	CMML	Normal
43	CMML	Normal
44	CMML	Trisomy 21
45	CMML-2	Trisomy 21
46	CMML-2	Normal
47	CMML-2	Normal
48	CMML-2	t(11;19)
49	CMML-MF	Normal
50	CMML, blast phase	Normal
51	CMML, blast phase	Normal
52	CMML, blast phase	Normal

aCML, atypical chronic myeloid leukemia; chr, chromosome; CMML, chronic myelomonocytic leukemia; ET, essential thrombocythemia; MDS/MPN, myelodysplastic/myeloproliferative neoplasms; MF, myelofibrosis; MPN, myeloproliferative neoplasm; PMF, primary myelofibrosis; PV, polycythemia vera; SM-AHNMD, systemic mastocytosis with associated clonal hematologic non–mast cell lineage disease.

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a rate of 17% for ET (2/12), 18% for CMML (3/17), 20% for SM-AHNMD (1/5), and 44% for PMF (7/16). The recurrent chromosomal abnormalities in the *JAK2*– MPN and MDS/MPN cases differed from those in the *JAK2*+ group. Trisomy 21 and 13q– were recurrent cytogenetic abnormalities in the *JAK2*– group, whereas only 1 case had 20q– and no case had trisomy 9. Of 3 cases with trisomy 21, 2 were identified at a stage of excess blast/blastic transformation, but there were no 13q– cases with excess blasts/blastic transformation. Chromosome 5 or chromosome 7 abnormality was also uncommon, seen in only 1 case with an abnormal chromosome 5 and 7q–. It is interesting that 1 case had t(8;9)/*PCM1-JAK2*, previously described in other hematologic malignancies, such as atypical chronic myeloid leukemia, AML, and T-cell lymphoma.[19-21]

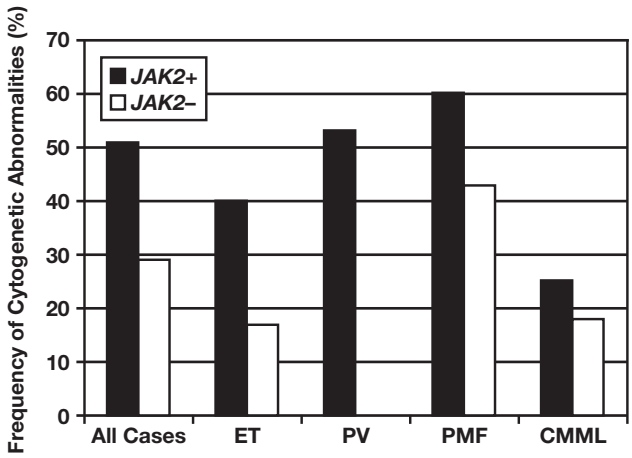

Figure 1 Comparison of the frequency of cytogenetic abnormalities between *JAK2*+ and *JAK2*– myeloproliferative neoplasm (MPN) and myelodysplastic/myeloproliferative neoplasm (MDS/MPN) groups. CMML, chronic myelomonocytic leukemia; ET, essential thrombocythemia; PMF, primary myelofibrosis; PV, polycythemia vera.

Comparison of Cytogenetic Features Between *JAK2*+ and *JAK2*– MPN and MDS/MPN

The overall prevalence of cytogenetic abnormalities in the *JAK2*+ group was significantly higher than that of the *JAK2*– group (23/45 [51%] vs 14/52 [27%]; $P < .05$). This trend was seen in each subtype of MPN and MDS/MPN **Figure 1**, even though the difference in each subtype did not reach statistical significance owing to the small sample. The rate of blastic transformation/excess blasts was higher in the *JAK2*+ group than that in the *JAK2*– group (15/45 [33%] vs 11/52 [21%]); it parallels the frequency of cytogenetic abnormalities.

When correlating with stage of the disease, our results indicate that the frequency of cytogenetic abnormalities continuously increased from a nonfibrotic stage to marrow fibrosis and to blastic transformation/excess blasts regardless of *JAK2* mutation status **Figure 2**.

Recurrent chromosomal aberrancies differed between the *JAK2*+ and *JAK2*– groups **Table 5**. Cytogenetic abnormalities of chromosomes 5, 7, 9, 12, 17, and 20 were more common in the *JAK2*+ group, whereas chromosome 13 abnormalities

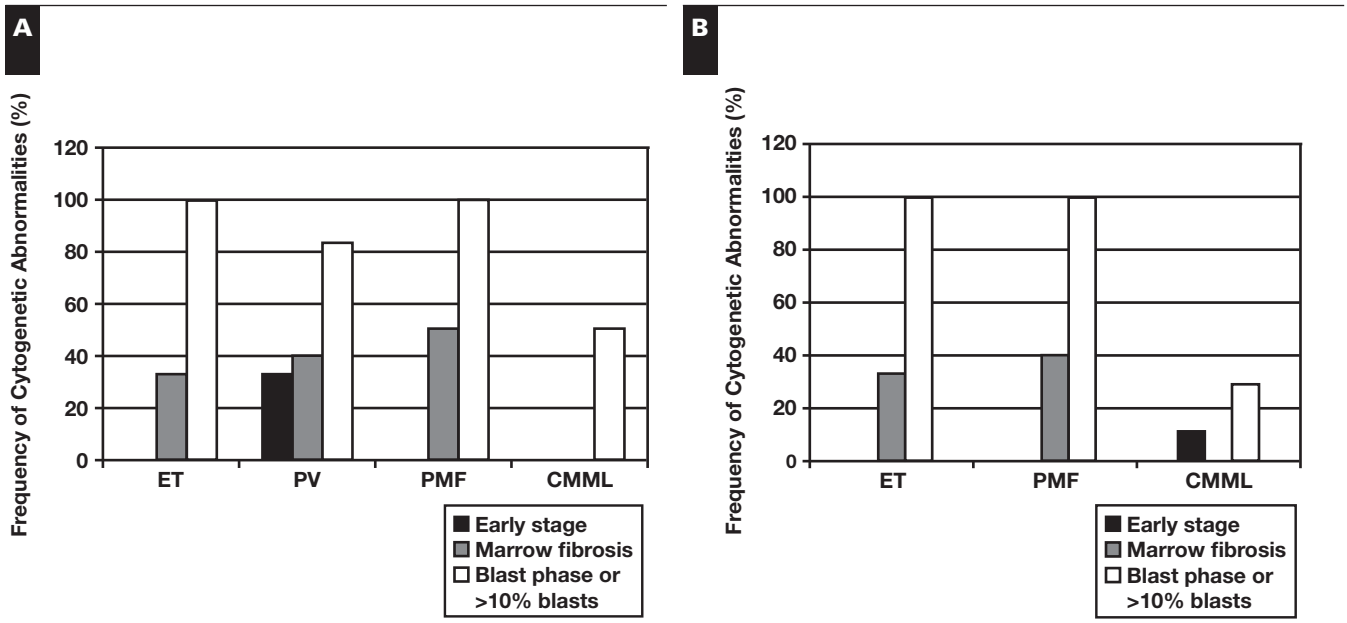

Figure 2 Frequency of cytogenetic abnormalities in different stages of myeloproliferative neoplasms (MPNs) and myelodysplastic/myeloproliferative neoplasms (MDS/MPNs). **A**, *JAK2*+ group. **B**, *JAK2*– group. CMML, chronic myelomonocytic leukemia; ET, essential thrombocythemia; PMF, primary myelofibrosis; PV, polycythemia vera.

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and trisomy 21 were more frequent in the *JAK2*– group. The 20q– was not identified in cases with blastic transformation (0/5), whereas abnormalities of chromosomes 5, 7, 9, and 17 and complex cytogenetics were unfavorable cytogenetic changes associated with blastic transformation **Table 6**. In 4 cases, a monosomal karyotype was found; 3 of these cases were identified in the *JAK2*+ group and 1 was identified in the *JAK2*– group. All cases with a monosomal karyotype were seen in patients with the blast phase of disease.

Discussion

The relationship between *JAK2*+ and *JAK2*– subtypes of MPN and molecular mechanisms of disease progression in the 2 subtypes is not clear. Several studies have indicated that a *JAK2* mutation is an unfavorable prognostic factor based on hematologic, clinical, and cytogenetic parameters; only a minority of studies reported different observations.[13,17,22-31]

The aim of our study was to systematically investigate the correlation of *JAK2* mutation status with cytogenetic abnormalities and the correlation of cytogenetic abnormalities with the stage of MPN and MDS/MPN. The frequencies and patterns of cytogenetic abnormalities of *JAK2*+ and *JAK2*– MPN and MDS/MPN were described and compared to determine whether specific cytogenetic abnormalities are more commonly associated with *JAK2* mutational status and whether these cytogenetic abnormalities yield prognostic information.

It is well-established knowledge that MPN and MDS/MPN disease progression to myelofibrosis and blast phase is correlated with a poor prognosis and shorter overall survival. The reported overall frequency of cytogenetic abnormalities in MPN varies between 3% and 40%.[15,24] Our cohort showed an overall rate of cytogenetic abnormalities of 38%, which is at the higher end of what has been reported. This variation seems related to the proportion of different stages of MPN and MDS/MPN included in the studies. Studies from mostly outpatient clinics usually report lower rates of abnormal cytogenetics because most patients are in an early phase of their disease in this setting. Our cohort includes a disproportionately larger number of patients with more advanced disease referred to the OHSU hematologic malignancy and bone marrow transplant center. For example, 33% (5/15) of patients with PV and 35% (6/17) with ET were admitted at the stage of post-PV or post-ET marrow fibrosis, and 40% (6/15) of patients with PV and 12% (2/17) with ET were admitted at the stage of excess blasts/blastic transformation. All of the aforementioned numbers are much higher than previously described in the literature, in which 10% to 15% of PV and 1% to 5% of ET cases reportedly evolve to marrow fibrosis[32,33]; and fewer than 5% of PV and fewer than 1% of ET[34] cases transform to blast phase.

In the present study, the lowest rate of cytogenetic abnormalities was in early stage PV and ET without marrow fibrosis or blastic transformation, 40% to 50% in myelofibrosis, and nearly 90% in blastic transformation/excess blasts. These findings are in accordance with published studies.[12,35] Our study further highlights that this trend is observed in *JAK2*+ and *JAK2*– groups and shows that cytogenetic abnormalities are more frequent in the *JAK2*+ group than in the *JAK2*– group (51% vs 27%; $P < .05$), which has not been reported by other groups using conventional cytogenetic analysis. Only recently, Tefferi et al[36] showed that changes of chromosome copy number were detected more often in *JAK2*+ ET and PMF cases by using oligonucleotide array comparative genomic hybridization. We demonstrated a greater frequency of blast phase disease in the *JAK2*+ than in the *JAK2*– cases (33% vs. 21%). Although the latter was not statistically significant, other studies have shown that *JAK2* mutation confers a worse prognosis using hematologic and clinical parameters **Table 7**.

Table 5
Comparison of the Numbers of Cytogenetic Abnormalities in *JAK2*+ and *JAK2*– Groups

Cytogenetic Abnormality	*JAK2*+	*JAK2*–	*P*
5q–, –5, abnormal 5	3	1	NS
7q–, –7	6	1	<.05
t(1;9) with extra derivative 9, +9, interstitial del 9, t(8;9)	5	1	<.05
t(2;12), t(5;12), t(10;12), t(12;17), 12q–, inv12, abnormal 12	5	2	NS
13q–, 13p–, trisomy 13	1	3	NS
17q–, i(17q), –17	3	0	<.05
20q–	4	1	NS
+21	1	3	NS
Monosomal karyotype	3	1	NS
Complex cytogenetics	4	3	NS

NS, not significant.

Table 6
Frequency of Blastic Transformation and Excess Blasts With Associated Cytogenetic Abnormalities in *JAK2*+ and *JAK2*– Groups*

Cytogenetic Abnormality	*JAK2*+	*JAK2*–	*JAK2*+ and *JAK2*–
17q–, i(17q), –17	3/3 (100)	0/0 (0)	3/3 (100)
Complex cytogenetics	4/4 (100)	2/3 (67)	6/7 (86)
Monosomal karyotype	3/3 (100)	1/1 (100)	4/4 (100)
5q–, –5, abnormal 5	3/3 (100)	0/1 (0)	3/4 (75)
t(1;9), +9	4/5 (80)	0/1 (0)	4/6 (67)
7q–, –7	4/6 (67)	0/1 (0)	4/7 (57)
+21	1/1 (100)	1/3 (33)	2/4 (50)
13q–, 13p–, trisomy 13	1/1 (100)	1/3 (33)	2/4 (50)
t(2;12), t(5;12), t(10;12), t(12;17), –12, 12p–, inv12, abnormal 12	3/5 (60)	0/2 (0)	3/7 (43)
20q–	0/4 (0)	0/1 (0)	0/5 (0)

* Data are given as number/total (percentage).

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Table 7
***JAK2* Mutations and Clinical Variables/Prognosis**

Reference	Patient Population	Clinical Association of *JAK2* Mutation
Kralovics et al,[5] 2005	PV, ET, PMF	V617F mutation associated with longer duration of disease and higher rate of fibrosis, hemorrhage, and thrombosis
Kittur et al,[13] 2007	ET	V617F mutation associated with increased hemoglobin level, lower platelet count, higher leukocyte count, venous thrombosis, older age, and worse survival (with exclusion of 2 outlier cases)
Tefferi et al,[14] 2006	PV	Patients homozygous for *JAK2* mutations (compared with heterozygotes and wild-type cases) showed increased hemoglobin level at diagnosis and increased rate of fibrotic transformation
Campbell et al,[22] 2006	PMF	V617F mutation associated with higher neutrophils and WBC counts and worse overall survival
Barosi et al,[23] 2007	PMF	V617F mutation associated with increased risk of splenomegaly and leukemic transformation
Wolanskyj et al,[25] 2005	ET	V617F mutation associated with advanced age, higher hemoglobin level and WBC counts, and transformation to PV but no association with fibrotic/leukemic transformation or inferior survival
Tefferi et al,[29] 2005	Primary and secondary myelofibrosis	V617F mutation associated with older age at diagnosis and history of thrombosis or pruritus but no prognostic significance
Tefferi et al,[30] 2008	PMF	V617F mutation associated with older age, platelet count >100 × 10^3/μL (100 × 10^9/L) and low peripheral blast count; V617F mutation *not* associated with thrombosis, overall survival, or leukemia-free survival; shortened overall and leukemia-free survival in patients with low V617F allele burden
Guglielmelli et al,[31] 2009	Post–PV MF and post–ET MF	All post–PV MF cases had $JAK2^{V617F}$ mutation compared with 27% of post–ET MF cases; post–PV MF cases had greater *JAK2* allele burden; no association between *JAK2* and transformation to acute myeloid leukemia

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

This study further stratified *JAK2*+ MPN and MDS/MPN cases using cytogenetic data. Chromosome 7 abnormalities were more often seen in cases with a *JAK2* mutation and conferred a worse prognosis with a higher rate of blastic transformation ($P < .05$). To the best of our knowledge, this is a unique observation, not previously reported.

By examining the pattern of cytogenetic abnormalities in *JAK2*+ and *JAK2*– MPN and MDS/MPN cases, we showed that chromosome 9 abnormalities and 20q– were also seen with higher frequency in patients with a *JAK2* mutation. Campbell et al[24] reported a similar observation. Our study also demonstrated that chromosome 9 abnormalities were more often seen in patients with blastic transformation in the *JAK2*+ group, with a *P* value less than .05 using univariate analysis compared with the cases without abnormal chromosome 9; however, this difference did not reach statistical significance in multivariate analysis. Sequential cytogenetic follow-up data were available for 2 of our cases with chromosome 9 abnormalities. In one case of PV with blast phase, the original clone demonstrated trisomy 9 already detected

Table 8
Cytogenetic Studies in MPN and MDS/MPN

Reference	Patient Population	Frequency of Cytogenetic Abnormalities (%)	Recurrent Cytogenetic Abnormalities
Bacher et al,[8] 2009	MDS, MPN, and MDS/MPN	MDS, 35.4; MDS/MPN, 27.9; MPN, 15	–Y, +8, del5q
Tefferi and Gilliland,[3] 2005	Myelofibrosis with myeloid metaplasia	46	13q–, 20q–, t(1;6), t(1;7), 11q–
Bacher et al,[15] 2005	Chronic myeloproliferative disorders	PMF, 40; PV, 35; CMML, 24; MPN, NOS, 21; ET, 3; HES, 7	Trisomy 8, trisomy 9, 20q–, monosomy 7
Gangat et al,[16] 2009	ET	7	Trisomy 9, trisomy 8, abnormalities of 1
Gangat et al,[17] 2008	PV	11-15	Trisomy 8, trisomy 9, 20q–, abnormalities of 1, –Y
Panani,[43] 2007	Philadelphia chromosome–negative chronic myeloproliferative disorders	PV and IMF, 30-40; ET, 5-6	20q–, 13q–, 12p–, +8, +9
Tefferi et al,[37] 2001	Myelofibrosis with myeloid metaplasia	48	20q–, 13q–, +8, +9, 12p–, abnormalities in 1 and 7
Strasser-Weippl et al,[41] 2005	IMF and secondary MF	56	–7/7q–
Strasser-Weippl et al,[42] 2006	Chronic myelofibrosis	56	20q deletions; 13q–

CMML, chronic myelomonocytic leukemia; ET, essential thrombocythemia; HES, hypereosinophilic syndrome; IMF, idiopathic myelofibrosis; MDS, myelodysplastic syndrome; MDS/MPN, myelodysplastic/myeloproliferative neoplasms; MF, myelofibrosis; MPN, myeloproliferative neoplasm; NA, not applicable; NOS, not otherwise specified; PMF, primary myelofibrosis; PV, polycythemia vera.

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in chronic phase, and an additional new deletion of 7q– was seen at the time of blastic transformation. For another case of CMML with blastic transformation, the original clone demonstrated a trisomy 9 at diagnosis that evolved into a tetraploid chromosome 9 at the time of transformation (Table 3, cases 18 and 45, respectively). The high frequency of chromosome 9 abnormalities detected in blastic transformation suggests that chromosome 9 abnormalities may be more likely associated with a worse prognosis.

In contrast, 13q– and trisomy 21 were more common in the *JAK2*– group in our series. Several groups have shown 13q– and 20q– to be associated with favorable outcomes in PMF,[2,15,37] but they do not address the relationship to *JAK2* mutation status. In addition, abnormalities of chromosomes 5, 7, and 17 were seen only in cases of myelofibrosis and blastic transformation in our study, suggesting these changes may be related to late-stage disease.

There are limited data on *JAK2* mutations in mastocytosis reported in the literature, and a recent article described 5 cases of systemic mastocytosis and coexisting PMF, 4 of which harbored a *JAK2* mutation.[38] One group showed no mutations in 28 cases of mastocytosis,[4] and Steensma et al[2] demonstrated a *JAK2* mutation in 2 of 8 cases of systemic mastocytosis, 1 of which had associated myelofibrosis and CMML**.** Our study found 2 of 7 SM-AHNMD cases had *JAK2* mutations. The variation of *JAK2* mutational status in mastocytosis is likely related to underling MPN rather than mastocytosis.

A monosomal karyotype is defined as 2 or more distinct autosomal monosomies or a single autosomal monosomy in the presence of 1 or more structural chromosomal abnormalities and has been shown to be associated with a poor prognosis in AML.[39,40] Consistent with these data, in our cohort, 4 cases had a monosomal karyotype (3 *JAK2*+ and 1 *JAK2*–), and all of these cases underwent blastic transformation or had excess blasts.

Review of the literature **Table 8**[3,8,15-17,37,41-43] revealed recurrent cytogenetic abnormalities in MPN and MDS/MPN, including 20q–, 13q–, 12p–, +8, +9, 7q–/–7, 5q–/–5, and i17.[15-17,29,37,41,42] However, correlation of *JAK2* mutation status and cytogenetic abnormalities with prognosis and clinical variables is not well established. It has been suggested that V617F– myeloproliferative neoplasms may represent an earlier phase of the same disease, with a subsequent acquisition of the *JAK2* mutation. A study by Kralovics et al[44] suggested the existence of "pre-*JAK2* alleles." Based on their findings, it has been proposed that the *JAK2* mutation is a late event in the evolution of MPN, arising in a background of other chromosomal abnormalities. Our data suggest that certain cytogenetic changes are more prone to *JAK2* mutation. Specifically, trisomy 9 and 20q– are more often seen in *JAK2*+ cases, whereas 13q– is more likely to be seen in *JAK2*– cases. These findings indicate that *JAK2*+ and *JAK2*– MPNs represent 2 independent genetic pathways with distinct patterns of cytogenetic aberrancies that might not overlap.

Our study highlights the combined role of testing for *JAK2* mutation and routine karyotyping in monitoring disease progression and stratifying risk in patients with MPN and MDS/MPN. Multicenter studies are needed to develop a cytogenetic-molecular–based risk stratification system for optimal targeted therapy and improved outcome of MPN and MDS/MPN, as already used in AML management.

Clinical Associations

Isolated chromosome 9 abnormalities seen only in MPN; higher frequency of –7 in MDS/MPN; isolated 5q(del), –7, and complex aberrations more common in MDS	
13q– and 20q– associated with better prognosis	
NA	
Abnormal cytogenetics associated with splenomegaly, tobacco use, venous thrombosis, and anemia but no impact on disease evolution or survival	
No association with worse outcome	
NA	
+8, 12p– associated with inferior survival; survival not adversely affected by 20q– or 13q–	
–7/7q– associated with worse prognosis but did not correlate with leukemic transformation	
Could not confirm better prognosis in 13q– and 20q– group	

From the Departments of [1]Pathology and [2]Molecular and Medical Genetics, Oregon Health & Science University, Portland; and [3]Clinical Pathology, William Beaumont Hospital, Royal Oak, MI.

Address reprint requests to Dr Fan: 3181 SW Sam Jackson Park Rd, Mail Code L471, Portland, OR 97239.

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