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Original contribution

Clinicopathological and molecular features of *SF3B1*-mutated myeloproliferative neoplasms☆,☆☆

Leonardo Boiocchi MD[a,b], Robert P. Hasserjian MD[a,b], Olga Pozdnyakova MD[b,c], Waihay J. Wong MD, PhD[b,c], Jochen K. Lennerz MD[a,b], Long Phi Le MD, PhD[a,b], Dora Dias-Santagata PhD[a,b], Anthony J. Iafrate MD, PhD[a,b], Gabriela S. Hobbs MD[b,d], Valentina Nardi MD[a,b,*]

[a]*Department of Pathology, Massachusetts General Hospital, 02114 Boston, MA, USA*
[b]*Harvard Medical School, 02114 Boston, MA, USA*
[c]*Department of Pathology, Brigham and Women's Hospital, 02114 Boston, MA, USA*
[d]*Division of Hematology/Oncology, Massachusetts General Hospital, Harvard Medical School, 02114 Boston, MA, USA*

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Summary The introduction of next-generation sequencing has broadened the genetic landscape of myeloproliferative neoplasms (MPNs) beyond *JAK2, MPL*, and *CALR*. However, the biological role and clinical impact of most other mutations are not well defined. We interrogated 101 genes in 143 *BCR-ABL1*-negative MPNs in chronic phase from 2 large institutions. We detected *SF3B1* mutations in 15 cases (10%) and set to investigate the clinical, morphologic, and molecular features of *SF3B1* mutated (*SF3B1*+) MPNs in comparison to *SF3B1* wild-type (*SF3B1*−) cases and to identify distinctive features with myelodysplastic/myeloproliferative neoplasms with ring sideroblasts (RS) and thrombocytosis, which can show partial clinical and morphological overlap with MPNs. *SF3B1*+ cases were enriched in primary myelofibrosis in both prefibrotic and fibrotic stage, but mutations of *SF3B1* seem to occur only as a late event in the fibrotic phase of essential thrombocythemia and polycythemia vera. *SF3B1*+ MPNs showed borderline lower hemoglobin but no other clinical or molecular differences compared to *SF3B1*− MPNs. Of note, RS were present only in a subset of *SF3B1*+ cases (4/10) without any other feature of erythroid or granulocytic dysplasia. Our results suggest that mutations in *SF3B1* are not a rare event in MPNs, especially in primary myelofibrosis and during late fibrotic stages of essential thrombocythemia and polycythemia vera, but are not associated with myelodysplastic progression. Careful examination of bone marrow and peripheral blood for morphologic dysplasia is crucial to reach the correct diagnosis and avoid a misdiagnosis of myelodysplastic/

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* Corresponding author at: Department of Pathology, Massachusetts General Hospital, 55 Fruit St, Room 219 Warren Bldg, Boston, 02114, MA, USA.
E-mail address: vnardi@partners.org (V. Nardi).

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myeloproliferative neoplasms with RS and thrombocytosis, a pitfall with potential prognostic and therapeutic implications.

1. Introduction

The introduction of high-throughput sequencing technologies in recent years has broadened our knowledge of the genetic landscape of myeloproliferative neoplasms (MPNs). Next-generation sequencing (NGS)–based assays are increasingly performed as part of routine workup when a myeloid neoplasm such as an MPN is clinically suspected. The role of *JAK2*, *MPL*, and *CALR* as driver oncogenes in MPNs that impact the disease biology, diagnosis, and prognosis has been well characterized [1-4]. Much less is known about the prevalence and clinicopathologic impact of mutations in other genes given that NGS-based sequencing data in MPNs have become available only recently [5-10]. Studies have shown that additional mutations are frequent in MPNs, with 75%-80% of primary myelofibrosis (PMF) [6] and more than 50% of polycythemia vera (PV) and essential thrombocythemia (ET) cases [7-10] carrying at least 1 mutation other than *JAK2*/*MPL*/*CALR*; overall, however, the mutation rate of any single gene is low. Mutated genes include those encoding for signaling molecules (*FLT3, CBL, KIT, NRAS, KRAS, PTPN11*), regulators of DNA methylation (*DNMT3A, TET2, IDH1, IDH2*), epigenetic regulators (*ASXL1, SETBP1, EZH2),* splicing factors *(SF3B1, SRSF2, U2AF1*, and *ZRSR2*), tumor suppressors (*TP53*), and myeloid transcription factors (*CEBPA*) [5-10]. The biological and clinical impact of these rarer molecular alterations in MPNs has not yet been fully characterized. Initial reports proposed that mutations of *ASXL1*, *SRSF2*, *CBL*, *KIT*, *RUNX1*, *SH2B3*, and *CEBPA* might have an independent negative prognostic impact on overall and leukemia-free survival and myelofibrotic progression [6,7,10,11]; only recently was a prognostic scoring system for PMF proposed that integrates the molecular profile [12].

Mutations of *SF3B1* (splicing factor 3B subunit 1), a component of the U2-small nuclear ribonucleoprotein complex, are uncommon in MPNs and are reported to occur in <10% of cases [6-8,10,13]. In contrast, this mutation is present in ≥80% of cases of MDS with ring sideroblasts (MDS-RS) and in MDS/MPN with RS and thrombocytosis (MDS/MPN-RS-T) and strongly correlates with presence of bone marrow RS in these diseases [14]. MDS/MPN-RS-T shares some clinical and morphologic features with MPNs because some MPN patients may similarly present with anemia and thrombocytosis. Distinction between *SF3B1*-mutated MPNs and MDS/MPN-RS-T is clinically relevant in terms of both disease prognosis and clinical management [15-17]; unlike "pure" MPNs, MDS/MPN-RS-T manifests significant dysplasia in the erythroid lineage [18]. *SF3B1* mutation can occur in clonal hematopoiesis of indeterminate potential, MDS, and acute myeloid leukemia (AML) [19,20] and appears to confer a favorable prognosis in MDS, but the significance of this mutation in MPNs is unknown. In this study, we describe and characterize the prevalence and the clinicopathologic features of *SF3B1*-mutated MPNs and compare them to *SF3B1*-WT MPNs.

2. Materials and methods

2.1. Case selection

Cases were identified by a search of the pathology database at 2 institutions: Massachusetts General Hospital (MGH) and the Brigham and Women's Hospital (BWH). All cases of MPN seen over a period of 4 years (2014-2017) were reviewed retrospectively, and 143 consecutive cases of *BCR-ABL1*–negative MPNs (excluding chronic eosinophilia leukemia/idiopathic hypereosinophilic syndrome) in chronic phase (36 from MGH, 107 from BWH) were identified. All cases in accelerated or blast phase (>10% bone marrow or peripheral blood blasts) were excluded. The study series included both patients diagnosed with MPN for the first time and patients with a known history of MPN receiving a follow-up bone marrow biopsy. Bone marrow biopsy, aspirate, and concurrent NGS-based panel testing results were available for all cases. After review of the clinical records and the pathologic findings, all cases were classified per the 2017 World Health Organization (WHO) classification [18,21,22]. The study was approved by the Institutional Review Boards of MGH and BWH.

2.2. Morphologic analysis

Hematoxylin and eosin (H&E)– and Giemsa-stained slides of marrow core biopsies and Wright-Giemsa–stained bone marrow aspirates and peripheral blood smears were reviewed for each case to confirm the diagnosis. Gomori's silver impregnation and Masson's trichrome stains were used to grade fibrosis according to the European Consensus Grading System [23]. An iron stain was performed on all *SF3B1*-mutated cases and all *SF3B1*-WT cases with an available unstained marrow aspirate slide, together with appropriate positive controls. Blasts were counted on marrow aspirates, touch preparations, and peripheral blood smears. In cases for which no smears or touch preparations were available (such as due to a dry tap), an immunohistochemical stain for CD34 was performed on the core biopsy to assess for any increase in blasts.

2.3. Cytogenetic analysis

Cytogenetic analysis was performed with trypsin-Giemsa banding techniques on bone marrow cells from aspirates obtained from the diagnostic bone marrow sample, and karyotype abnormalities were described per the International System for Human Cytogenetic Nomenclature [24].

2.4. Molecular studies

Targeted DNA sequencing was performed via Rapid Heme panel (n = 133) (BWH), a 95-gene panel based on the TruSeq Custom Amplicon kit (Illumina, San Diego, CA) [25], or via Heme SNaPshot NGS v1 assay (MGH), based on the Trusight Myeloid Sequencing Panel (Illumina, San Diego, CA) targeting 54 genes (n = 10). Both assays use the same methods for target enrichment and share 48 gene targets. A total of 101 distinct genes were investigated between the 2 assays (for the complete list of target genes, see Supplementary Table). Based on internal validation studies, there is >97% concordance of mutations identified in samples run by both assays (data not shown). An Illumina (San Diego, CA) MiSeq sequencer was used with 2 × 151–base pair paired-end chemistry. The sequencing results were analyzed with the Illumina BaseSpace TruSeq Amplicon v2.0.0 somatic variant caller (0.5% threshold, read stitching on) and, at MGH, via CIDer (Variant Effect Predictor v83 and target coverage analysis) [25]. The Rapid Heme panel assay had been validated to detect SNV and indel variants at any allele frequency with >10 supporting reads, whereas the Heme SNaPshot–validated allelic frequency was 10%, with lower allelic frequencies being reported for target regions with sufficient read coverage.

2.5. Statistical analysis

All statistical analyses were performed using the SPSS statistics 20 software (SPSS, Chicago, IL). $P < .05$ was considered significant for all statistical tests, and all tests were 2-sided. Difference in continuous variables was evaluated with *t* test or analysis of variance test. Fisher exact test or Pearson χ^2 test was used to evaluate proportions. The co-mutational plot was drawn in R Studio (RStudio Team (2016). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, URL http://www.rstudio.com/) using the "PHeatMap" package.

3. Results

3.1. Cohort

This bi-institutional study analyzed the features of a series of 143 patients with *BCR-ABL1*–negative MPN in chronic phase for whom targeted DNA sequencing studies had been performed. Fifty-one (35%) cases were classified as PMF; 39 (27%) as ET; 32 (22%) as PV; and 21 (15%) as MPN, unclassifiable (MPN-U). Among the PMF cases, 16 (11% of all cases) were further defined according to 2017 WHO classification criteria as early/prefibrotic PMF (p-PMF), whereas the remaining 35 (24%) were in advanced fibrotic stage (f-PMF). Ten cases of ET (7% of all cases) and 11 cases of PV (8% of all cases) were classified as post-ET and post-PV myelofibrosis (MF), respectively. The clinical details of all cases are summarized in Table 1. Eighty-seven (61%) patients received a new diagnosis, whereas 57 (39%) had a prior history of MPN.

3.2. Morphologic and flow cytometry analysis of bone marrow

Histological review of bone marrow samples showed the distinctive features characteristic of each MPN entity as described in the updated 2017 WHO classification [22], including megakaryocytic proliferation associated with

Table 1 Summary of the clinical data of the entire MPN study cohort (n = 143)

	PMF			ET			PV			MPN-U
	p-PMF (n = 16)	f-PMF (n = 35)	Total (n = 51)	ET (n = 29)	Post-ET MF (n = 10)	Total (n = 39)	PV (n = 21)	Post-PV MF (n = 11)	Total (n = 32)	(n = 21)
Age (y)	68.6	67.0	67.5	52.2	66.1	55.8	58.8	68.8	61.5	63.9
Sex	9 M/7 F	26 M/9 F	35 M/16 F	13 M/16 F	4 M/6 F	17 M/22 F	12 M/9 F	2 M/9 F	14 M/18 F	10 M/11 F
Hg (g/dL)	11.7	9.5	10.2	13.1	10.2	12.4	14.4	10.3	12.9	11.2
MCV (fL)	90.5	86.5	87.7	88.8	90.5	89.2	82.4	97.0	87.7	88.7
WBC ($\times 10^9$/L)	18.4	11.0	13.3	9.2	25.3	13.4	18.8	7.0	14.5	26.9
Neutrophils ($\times 10^9$/L)	14.0	7.1	9.2	6.6	14.9	8.4	14.0	4.8	10.8	15.8
Monocytes ($\times 10^9$/L)	0.9	0.4	0.6	0.8	0.6	0.8	0.8	0.3	0.6	7.8
Marrow blasts %	<1%	1.3	1%	<1%	<1%	<1%	<1%	<1%	<1%	<1%
Platelets ($\times 10^9$/L)	522	234	324	606	345	540	377	203	316	345

NOTE. Mean values are reported, unless otherwise specified.
Abbreviations: F, female; Hg, hemoglobin; M, male; MCV, mean corpuscular volume.

Table 2 Distribution of *JAK2*/*CALR*/*MPL* driver mutations in the MPN study cohort

	PMF (n = 51)	ET (n = 39)	PV (n = 32)	MPN-U (n = 21)	Total MPNs (n = 143)	*P*
JAK2	36 (71%)	22 (56%)	29 (91%)	13 (62%)	100 (70%)	.01
CALR	6 (12%)	17 (44%)	0	4 (19%)	27 (19%)	<.001
MPL	5 (10%)	1 (3%)	0	1 (5%)	7 (5%)	NS
TN	4 (8%)	1 (3%)	3 (9%)	2 (10%)	10 (7%)	NS

Abbreviations: NS, not significant; TN: triple negative.

characteristic atypical megakaryocyte morphology and variable cellularity, megakaryocyte histotopography, and granulocytic and erythroid proliferation, according to the specific MPN type. After careful review, no neutrophilic or erythroid dysplasia (>10% of cells in any hematopoietic lineage) was appreciated in any of the cases upon histologic review of the bone marrow core sections or Wright-Giemsa–stained bone marrow aspirate smears or peripheral blood smears. Bone marrow blasts ranged from <1% to 5%. The detailed morphologic features of a large subgroup of cases (107 cases from BWH) included in the present study were previously described [26]. Flow cytometry of the bone marrow revealed no clonal B-cell or abnormal T-cell populations and <5% blasts in all cases.

3.3. Cytogenetic analysis

A complete karyotype was obtained in 121 cases (85%). Eighty (66%) showed a normal karyotype. Among the remaining 41 cases (34%), the most common alterations were del (20q) (n = 9), del(13q) (n = 6), and trisomy 9 (n = 6). A

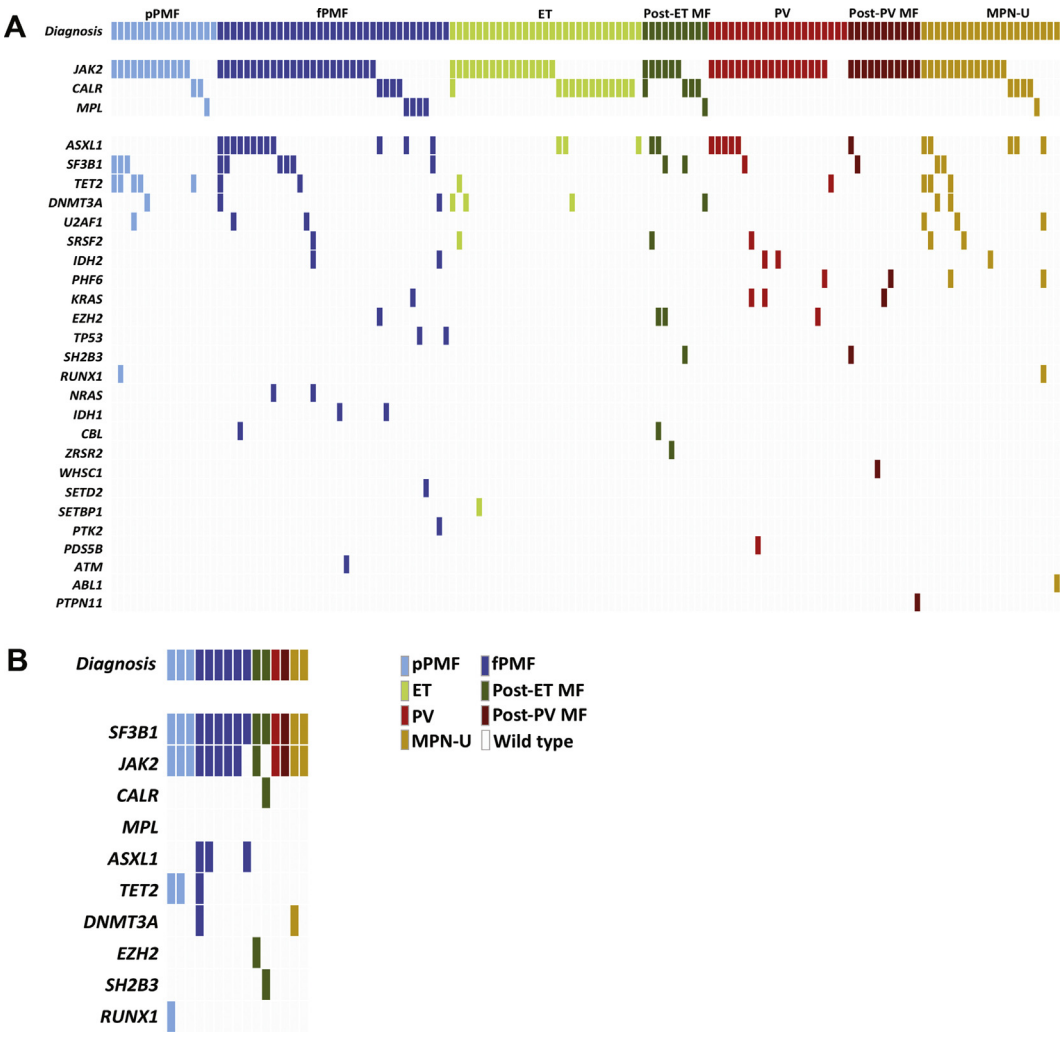

Fig. 1 A, Co-mutational plot of all genes investigated in the whole series of MPNs included in the study (n = 143). B, Co-mutational plot of the *SF3B1*-mutated MPNs (n = 15); only genes mutated at least once were included.

Table 3 Clinical, molecular, and morphologic features of the MPN cases with *SF3B1* mutations

Case #	Age (y)	Sex	Diagnosis	Duration of MPN (y)	Mutations; VAF %		Fibrosis	Marrow dysplasia	Cytogenetics	Hb (g/dL)	MCV (fL)	WBC (10^9/L)	ANC (10^9/L)	Platelets (10^9/L)	Marrow RS %	FU
					JAK2/CALR/MPL	*SF3B1*										
1	79	F	Post-ET MF	33	*CALR*; 40	H662D; 3	MF-3	No	Normal	9.5	91.1	3.6	2.6	133	0.0	Alive
2	76	F	MPN-U	8	*JAK2*; 99	K666 N; 43	MF-2	No	N/A	7.6	103.5	19.1	13.4	352	n/a	Dead
3	66	F	MPN-U	13	*JAK2*; 97	H662Q; 44	MF-3	No	Normal	7	89.4	79.0	21.3	454	n/a	Alive
4	53	M	f-PMF	5	TN	T663I; 3	MF-2	No	46XY,del(11)(q22.1q24.3) [12]	8.7	95.4	1.8	0.8	116	n/a	Dead
5	69	F	f-PMF	4	*JAK2*; 75	K700E; 41	MF-3	No	46,XX,add(4)(p1?5) [2]	8.3	85.5	12.1	9.9	154	0.0	Dead
6	66	M	f-PMF	2	*JAK2*; 73	K700E; 37	MF-3	No	Normal	7.5	93.2	7.9	4.2	328	25.0	Alive
7	60	M	f-PMF	8	*JAK2*; 49	K666Q; 22	MF-3	No	Normal	8.5	88.9	3.1	1.9	114	n/a	Alive
8	47	F	PV	7	*JAK2*; 44	K666 N; 23	MF-1	No	Normal	15.7	84.5	14.2	10.1	618	0.0	Alive
9	74	F	Post-PV MF	15	*JAK2*; 56	K666 N; 5	MF-2	No	48XX,+8,+9	9.8	102	7.4	4.9	77	n/a	Alive
10	65	M	p-PMF	0	*JAK2*; 43	K666 N; 46	MF-1	No	Normal	12.7	81	11.5	7.4	925	30.0	Alive
11	74	M	p-PMF	5	*JAK2*; 5	K666 N; 47	MF-1	No	Normal	11.2	95.4	39.4	25.6	1294	0.0	Alive
12	85	M	f-PMF	6	*JAK2*; 48	K666 T; 40	MF-3	No	n/a	9.6	97	4.8	3.5	470	13.0	Alive
13	71	M	f-PMF	7	*JAK2*; 79	K666 N; 46	MF-2	No	46,XY,del(20)(q11.2)[cp19]/46,XY [1][cp19]/46,XY [1]	14.8	99	17.3	13.7	76	0.0	Alive
14	53	F	Post-ET MF	34	*JAK2*; 80	K700E; 37	MF-3	No	Normal	8.9	84	14.0	10.2	400	8.0	Alive
15	75	M	p-PMF	0	*JAK2*; 59	K666 N; 47	MF-1	No	Normal	12.2	86.4	39.9	35.9	807	0.0	Alive
Mean	67.5			9.8						10.1	91.8	18.3		421.2	7.6	

NOTE. Mean values are reported, unless otherwise specified.
Abbreviations: ANC, absolute neutrophils count; FU, follow-up.

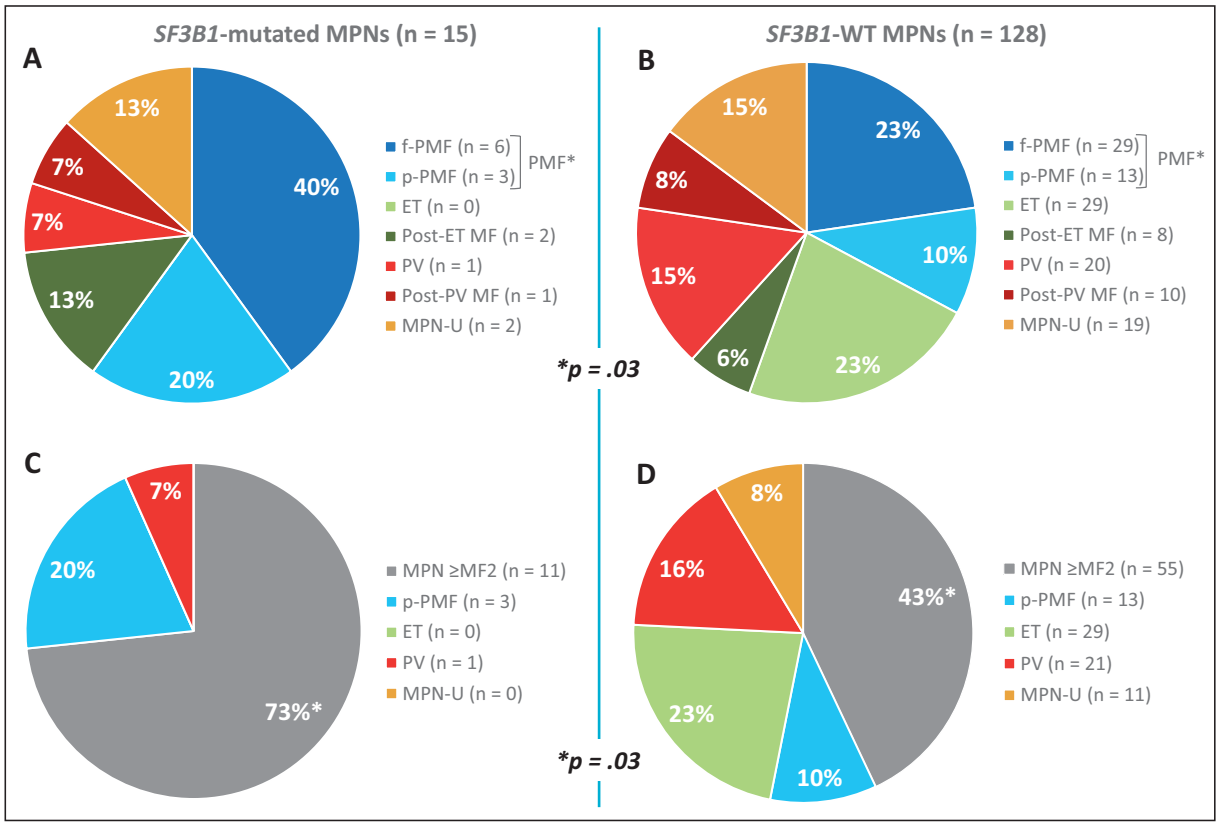

Fig. 2 *SF3B1* - mutated and *SF3B1*-wild type MPNs. The *SF3B1*-mutated group (A; n = 15) showed higher percentage of PMF cases, including both p-PMF and f-PMF, than the cohort of *SF3B1*-WT MPNs (B; n = 128; *P* = .03). However, the distribution of p-PMF and f-PMF cases in the 2 groups was similar. No ET in thrombocythemic phase was mutated. When aggregating all cases with fibrosis ≥MF2, either primary or secondary, the proportion of cases showing fibrosis ≥MF2 was significantly higher among the mutated cases (C) than among *SF3B1* wild-type MPNs (D) (*P* = .03; cases with MF ≥ 2 are highlighted in gray).

subset of cases showed single alterations which are often associated with myelodysplastic syndromes, including del(11q) (n = 3) and del(5q) (n = 2), but the peripheral blood count features and absence of morphologic dysplasia excluded the diagnosis of MDS or MDS/MPN.

3.4. Molecular analysis

One hundred cases (70%) were characterized by *JAK2* mutation, 27 (19%) by *CALR* mutation, and 7 (5%) by *MPL* mutation. Ten patients (7%) were "triple negative," (TN) having unmutated *JAK2, CALR*, and *MPL*. The distribution of the driver mutations among the different MPN entities was in line with data reported in literature and is detailed in Table 2. Mutations in 25 additional genes were identified in 77 of 143 cases (54%). Only 4 cases (3%, representing 4 of the 10 TN cases) did not show any mutation. The mutational load (calculated as the average number of mutations in each case) was not significantly different between the different types of MPNs or between fibrotic (MF grade 2-3) and nonfibrotic (MF grade 0-1) cases. However, f-PMF cases showed a borderline higher average number of mutations compared to ET cases (*P* = .053). Regarding specific mutation, *ASXL1* showed a significant association (*P* = .01) with f-PMF compared to p-PMF (Fig. 1). The co-mutational status for all cases is illustrated in detail in Fig. 1A. *SF3B1* was mutated in 15 cases (10%) and did not show any significant difference in mutation frequency among the MPN subtypes (*P* = .2).

3.5. *SF3B1*-mutated cases

All *SF3B1* mutations occurred in mutational hot spots at codons 662, 663, 666, or 700 (Table 3). The mean variant allele frequency for *SF3B1* mutations was 7.6% (range, 3%-47%). Thirteen of 15 cases (86%) showed concurrent *JAK2*V617F mutation, 1 case (7%) had a *CALR* exon 9 deletion (D373fs), and 1 case (7%) was triple negative for *JAK2*, *CALR*, and *MPL*. Based on the relative variant allelic frequencies (VAFs), the *SF3B1* mutation occurred as a late event in cases 1 and 9, as it is subclonal relative to the *JAK2* or *CALR* mutation, respectively (Table 3). However, in the remaining cases, the mutational hierarchy is less clear, as the VAF of *JAK2* V617F could be affected by copy-neutral loss of heterozygosity, a common phenomenon in advanced *JAK2*+ MPNs [27], which our clinical assays are not able to assess. Eight cases (53%) showed additional concurrent mutations,

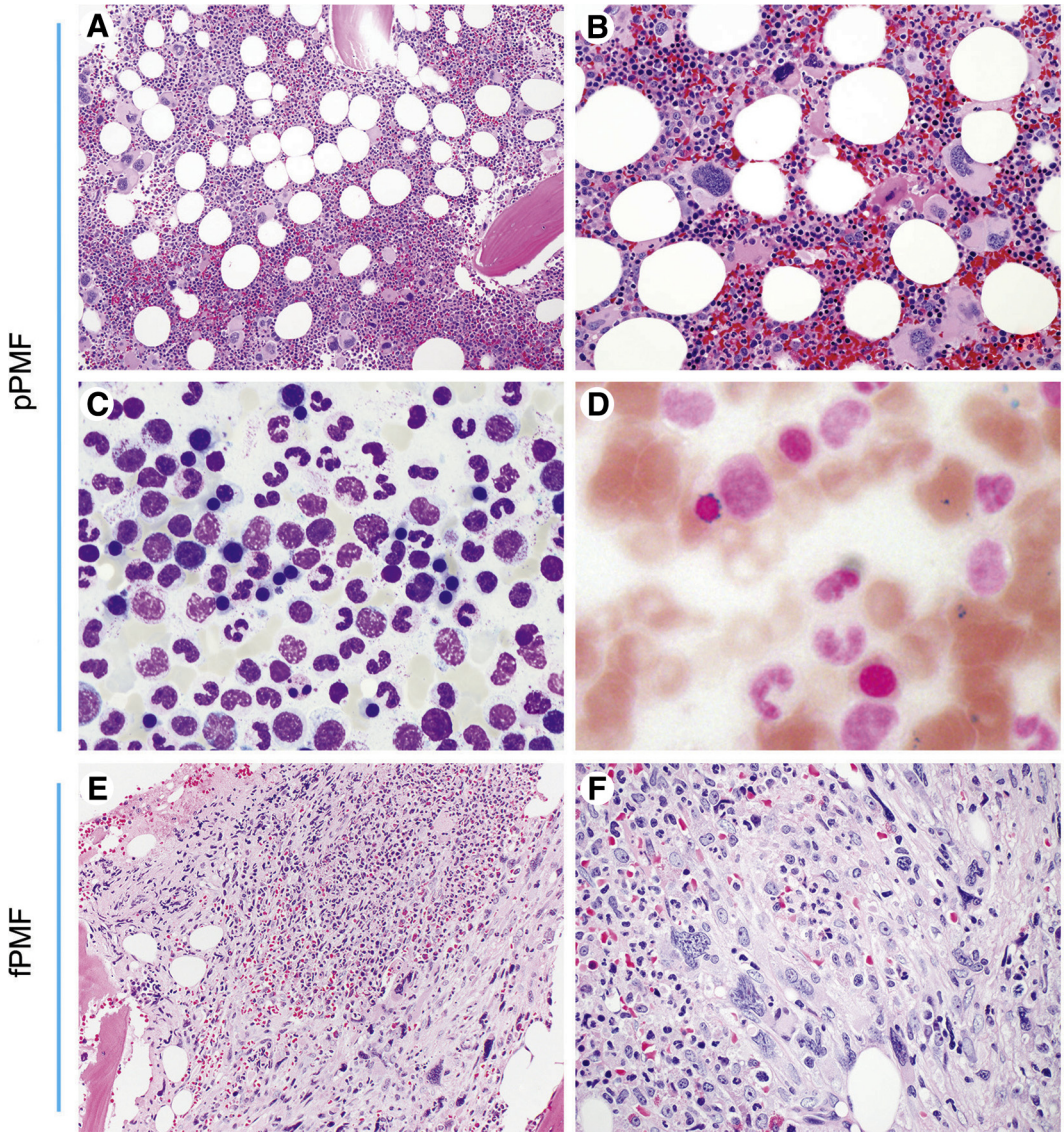

Fig. 3 Morphologic features of *SF3B1*-mutated MPN. Most of the cases (12/15) bearing an *SF3B1* mutation were PMF or post-PV or post-ET MF or MPN-U with MF2 fibrosis. All cases showed classic morphologic features of MPNs, with no overt dysplasia apparent in the marrow core biopsy, aspirate smears, or peripheral blood smears (A-D, case 10, p-PMF; E-F, case 13; A: H&E, original magnification ×4; B: H&E, ×20; C: bone marrow smear, ×100; E: H&E, ×4; F: H&E, ×40). D, The presence of *SF3B1* was associated with the presence of RS in a subset of cases.

including *ASXL1* (n = 3), *TET2* (n = 3), *DNMT3A* (n = 2), *EZH2*, *SH2B3*, and *RUNX1* (n = 1 for each) (Fig. 1B).

The *SF3B1*-mutated MPNs included PMF (n = 9, 60%, 3 p-PMF and 6 f-PMF), post-ET MF (n = 2; 13%), post-PV MF (n = 1; 7%), PV (n = 1; 7%), and MPN-U (n = 2, 13%; 1 with MF2 and the other 1 with MF3 fibrosis) (Fig. 2). Thirteen of 15 cases (87%) had a prior diagnosis of MPN, with a duration ranging between 2 and 34 years, whereas only 2 cases (both p-PMF, #10 and #15) were initial diagnoses. For cases 9 and 14, the diagnoses of post-ET and post-PV MF were confirmed after morphological review of the original marrow biopsies and confirmation of the prior diagnoses of PV and ET, respectively. For case 1, clinical records were carefully reviewed, and the clinical diagnosis of ET appeared to be appropriate based on the long history (>25 years) of isolated and asymptomatic thrombocytosis without polycythemia, which would be exceptionally rare for prefibrotic PMF (Table 3). The proportion of PMF cases in the *SF3B1*-mutated MPN group was significantly higher than among *SF3B1*-WT MPN (P = .03; Fig. 2). However, the distribution of p-PMF and f-PMF in the 2 cohorts was similar (P = .7). Among the ET cases, only post-ET MF cases showed mutations in *SF3B1*, whereas no ET cases in thrombocythemic phase were *SF3B1* - mutated. When considering fibrosis grade among all cases, there was a significantly higher proportion of cases with MF2-3 in the *SF3B1*-mutated group (P = .03, Fig. 2).

Morphologic examination of the marrow core biopsies and aspirate smears revealed features characteristic of each

Table 4 Comparison of clinical variables between *SF3B1*-mutated cohort (n = 15) and the *SF3B1* wild-type MPNs (n = 128)

	SF3B1-mutated MPNs (n = 15)	*SF3B1*-WT MPNs (n = 128)	*P*
Mean	67.5	61.9	NS
Sex	8 M/7F	68 M/60F	NS
PMF (n; % of total cases)	9; 60%	42; 33%	**.03**
ET (n; % of total cases)	2; 13%	37; 29%	NS
PV (n; % of total cases)	2; 13%	30; 23%	NS
MPN-U (n; % of total cases)	2; 13%	19; 15%	NS
Fibrosis ≥ MF2 (n; % of total cases)	11; 73%	55; 42%	**.03**
Hg (g/dL)	9.8	11.7	**.01**
MCV (fL)	92.2	87.7	NS
WBC ($\times 10^9$/L)	18.1	14.0	NS
Neutrophils ($\times 10^9$/L)	10.8	9.3	NS
Monocytes ($\times 10^9$/L)	0.7	0.8	NS
Marrow blasts %	<1%	<1%	NS
Platelets ($\times 10^9$/L)	343	389	NS

NOTE. Mean values are reported, unless otherwise specified.

assigned MPN subtype in all cases (Fig. 3). Megakaryocytes were increased and cytologically atypical in all cases. In PMF cases, the megakaryocytes showed hyperchromatic, bulbous, and naked nuclei and were grouped in loose and dense clusters. In ET cases, many megakaryocytes displayed hyperlobulated nuclei, whereas in PV, they were polymorphous, including large and smaller elements. Significant (>10%) dysplasia of the myeloid or erythroid lineages was not appreciated in any cases. Given the well-known association between mutations of *SF3B1* and the presence of RS in the bone marrow [28], an iron stain was performed on the 10 *SF3B1*-mutated cases for which an aspirate smear was available. RS were present in 4 cases (40%), representing 8%-30% of the nucleated erythroid elements (overall mean: 7.6%; mean among cases with RS: 19%; Fig. 3 and Table 3). In the remaining 6 cases, variable amounts of storage iron were present, but no RS were seen (Table 3). In 3 cases (#6, #12 and #14), the RS were seen in follow-up samples of patients who had received a prior diagnosis of MPN between 2 and 34 years earlier. Cases with RS showed a *SF3B1* variant allele frequency ranging between 37% and 46%. There was no correlation between the variant allele frequency or the mutation codon of *SF3B1* and the presence of RS. Two cases (case 3, MPN-U and case 12, f-PMF) were particularly interesting because they showed a combination of significant anemia (hemoglobin <10 g/dL) and thrombocytosis (platelet count >450 × 10^9/L) together with presence of RS. The combination of these features, together with the presence of an *SF3B1* mutation, could raise the differential diagnosis of MDS/MPN-RS-T. However, both patients had long-standing clinical features of a myeloproliferative neoplasm. Case 3 was that of a 66-year-old woman diagnosed in 2003 with asymptomatic isolated thrombocytosis (platelets >600 × 10^3/dL) and hemoglobin within normal limits. She was initially treated with anagrelide for 2 years until platelets started decreasing and the drug was then suspended. A first bone marrow biopsy performed in 2007 showed significant fibrosis (MF-2), and in 2014, she developed transfusion-dependent anemia, leukocytosis, and symptomatic splenomegaly. She was started on ruxolitinib and hydroxyurea in 2014, and at last follow-up in 2016, splenomegaly had decreased, and white blood cell count (WBC) was within normal limits. Case 12 was an 85-year-old man initially found to have isolated marked thrombocytosis in 2012 (900 × 10^3/dL), with hemoglobin within normal limits. He was started on hydroxyurea and aspirin but after 4 years developed significant anemia (7.5 g/dL) and fatigue with splenomegaly. A bone marrow biopsy was then performed for the first time, revealing significant fibrosis (MF-3). The patient was then started on ruxolitinib and was still transfusion dependent at last follow-up in 2018.

Thirty cases of MPNs from the *SF3B1*-WT group with an available unstained marrow aspirate smear (11 ET, 1 post-ET MF, 2 MPN-U, 7 PMF, 2 post-PV MF, and 7 PV) were also stained for iron and showed variable amounts of storage iron but no RS ($P < .01$ for the presence of RS compared to the *SF3B1*-mutated MPN cohort).

Comparison of clinical variables between *SF3B1*-mutated and *SF3B1*-WT groups revealed that *SF3B1*-mutated cases had lower hemoglobin levels (9.8 vs 11.8 g/dL; $P = .01$), with no significant differences in age or sex distribution, mean corpuscular volume, WBC and differential, marrow blasts, or platelet count. Considering only the subset of cases with MF2-3 (11 *SF3B1*-mutated cases and 55 *SF3B1*-WT cases) revealed a trend toward lower hemoglobin in the *SF3B1*-mutated cases (8.8 vs 9.9 g/dL, respectively, $P = .07$) but no significant differences between the 2 groups. Details on the comparison between *SF3B1*-mutated and control cases are summarized in Table 4.

4. Discussion

Targeted NGS panels are becoming an integral component of clinical management when a hematopoietic neoplasm is

suspected. Since the discovery of driver mutations in the *JAK2*, *MPL*, and *CALR* genes in *BCR-ABL1*–negative MPNs [1-4], there have been extensive efforts to characterize their clinical impact [29]. However, less is known about the frequency and the role of other mutations in these MPNs. The mutational landscape of MPNs has been unveiled only recently through publications of NGS studies of large MPNs cohorts [5-8]. Overall, these studies have suggested that MPNs are characterized by a relatively weak hypermutable state, at least until the disease progresses to blast phase [30]. In 1 recent publication, only 20% of PMF cases showed 3 or more mutations in addition to *JAK2, MPL*, or *CALR*, with *ASXL1*, *TET2*, *SRSF2*, and *U2AF1* being the most commonly mutated genes among the 27 genes investigated [6]. In PV and ET, almost 50% of cases harbored additional mutations, most commonly in *ASXL1*, *TET2*, *SH2B3*, and *DNMT3A* [7].

We interrogated the mutational status of a total of 101 distinct genes included in 2 clinically validated NGS-based panels on a combined population of 143 *BCR-ABL1*–negative MPNs consecutively seen over 4 years at 2 major referral centers. The combination of Rapid Heme and Heme SNaPShot assays represents the largest clinically validated panel of genes related to hematopoietic neoplasms investigated thus far in a population of MPNs. Among the earlier studies, only Tenedini et al [5] and Lundberg et al [9] used larger panels (1400 and 104 genes, respectively), whereas the remaining groups [6-8,10] investigated a more limited number of target genes (ranging between 27 and 33).

The most commonly mutated genes across our cohort of MPNs were *ASXL1*, *SF3B1*, *TET2*, and *DNMT3A*. Mutations of *ASXL1*, *TET2*, and *DNMT3A* have already been reported as the most commonly encountered mutations in clonal hematopoiesis of indeterminate potential [19,20] as well as in myeloid neoplasms including MPNs [5-8]. Data available in literature about the prevalence of *SF3B1* mutations in *BCR-ABL1*–negative MPNs are contradictory. In the first article applying high-throughput sequencing to 20 MPN cases, no mutations in *SF3B1* were found [5]. Subsequent studies showed that *SF3B1* mutations were more frequent in PMF (10% of cases) than in PV or ET (3% and 5%, respectively) [6,7]. Another study described a higher prevalence of mutations of splicing genes in PMF compared to ET and PV, although mutations of *SF3B1* were rare in PMF and ET and absent in PV [8]. In our series, the overall prevalence of *SF3B1* mutations among all MPNs was 10% and even higher in PMF (18%), which is more frequent than previously reported in literature. The proportion of PMF cases in the *SF3B1*-mutated group was significantly higher than in *SF3B1*-WT MPN, and *SF3B1* mutation was also significantly more common in cases with MF2-3 fibrosis overall; conversely, *SF3B1* mutations were not found in ET in thrombocythemic phase and were rare in PV cases in polycythemic phase. Our results are in line with the scarce data present in literature regarding *SF3B1* mutations in ET and PV. Senin et al recently described the association between *SF3B1* mutations and progression to post-PV MF in a series of PV patients with serial bone marrow samples [10]. Another study associated the presence of *SF3B1* mutations with decreased myelofibrosis-free survival in ET patients, although it failed to confirm a correlation between *SF3B1* mutations and myelofibrotic progression in PV [7]. The high percentage of post-ET and post-PV cases that lacked *SF3B1* mutations in our study suggests that mutations in *SF3B1* represent a late secondary event in only a subset of ET and PV cases [31]. However, in the single mutated case of post-PV MF in our cohort, *SF3B1* was the only mutated gene besides *JAK2*. In PMF, *SF3B1* mutations are more common than in ET and PV and also seem to occur earlier, as they were found with similar frequency in both p-PMF and f-PMF, raising the possibility that *SF3B1* mutations may not play a significant role in the fibrotic evolution of PMF. In line with this conclusion is the known rare occurrence of fibrosis in MDS/MPN-RS-T and MDS-RS, myeloid neoplasms in which mutations in *SF3B1* are early driver events [18,21]. In our data set, *ASXL1* mutations, in line with previous reports, showed a significant association with f-PMF [32].

Only 1 prior study by Lasho et al [13] specifically described the clinicopathologic features associated with *SF3B1* mutation in PMF. In a cohort of 155 PMF cases, 10 showed *SF3B1* mutations, a prevalence of 6.5%. *SF3B1*-mutated cases did not reveal significant differences in peripheral counts or other clinical parameters when compared with wild-type cases, but the mutation was invariably associated with high percentages of RS. We observed a higher proportion of *SF3B1*-mutated PMF cases (18%) than previously published studies [5,6,8,33]. We also showed for the first time that mutated cases appear to be equally distributed between p-PMF and f-PMF, although the number of cases was relatively small, and this observation would require further validation. In addition, in contrast with the Lasho et al study, our results suggest that only a subset of *SF3B1*-mutated cases showed RS, and thus, the absence of RS does not exclude an *SF3B1* mutation in MPN. Furthermore, we report for the first time the results of a careful morphologic review of *SF3B1*-mutated MPN cases in an attempt to identify any distinctive features. We saw no evidence of dysplasia (except for the presence of RS in a subset) in the *SF3B1*-mutated cases. Even in the setting of advanced fibrosis and leukoerythroblastosis, no dysgranulopoiesis was seen. A comparison of complete blood counts between *SF3B1*-mutated and wild-type cases revealed lower hemoglobin levels among the mutated cases.

It is clinically relevant to correctly distinguish *SF3B1*-mutated MPNs from MDS/MPN-RS-T. Although patients with MDS-RS or MDS/MPN-RS-T are generally thought of as having a relatively indolent course, patients with PMF may behave more aggressively; some PMF cases that progress or are refractory to conventional therapies may require hematopoietic cell transplantation [34]. Based on our results, this differential diagnosis might be a consideration, especially in cases of p-PMF that not infrequently present with marked thrombocytosis and borderline anemia. In 2 cases in our series (#3 and #12), significant anemia (hemoglobin <10 g/dL) was associated with thrombocytosis (platelet count >450 x 10^9/L).

The combination of these 2 abnormal parameters, together with the presence of an *SF3B1* mutation, could raise the differential diagnosis of MDS/MPN-RS-T. However, no RS were present in case 3, and RS were only 13% of erythroid cells in case 12, less than the WHO threshold of 15% required to diagnose MDS/MPN-RS-T (even in the setting of *SF3B1* mutation). Moreover, features of erythroid dysplasia (aside from the RS), typically present in MDS/MPN-RS-T, were absent [18]. None of the cases with *SF3B1* mutations in our series fulfilled the criteria for a diagnosis of MDS/MPN-RS-T: all cases lacked morphologic dysplasia, and when anemia and thrombocytosis were present (as in cases 3 and 12), there had been an established diagnosis of MPN-U and PMF, respectively, for several years. Case 10 presented de novo with an *SF3B1* mutation accompanied by marked thrombocytosis and 30% RS. However, it lacked morphologic erythroid and granulocytic dysplasia and anemia. Of note, the association of *SF3B1* mutations with increased reticulin fibrosis is not sufficient by itself to exclude the diagnosis of MDS/MPN-RS-T, as a proportion of cases of MDS/MPN-RS-T can present with reticulin fibrosis [18].

A very recent study on a large series of MPNs with thorough molecular characterization [35] has associated the presence of mutations in spliceosome genes, including *SF3B1*, with myelofibrosis and worse prognosis compared to cases with only heterozygous *JAK2* mutations. The enrichment in myelofibrotic cases among *SF3B1*-mutated MPNs in our cohort seems to reinforce the suspicion that *SF3B1* mutations might be clinically relevant in MPNs and purport a less favorable prognosis, whereas in MDS, they are generally associated with a better prognosis [15]. Our findings underline also how morphological analysis in patients with persistent significant myeloproliferation complements models exclusively based on molecular and clinical data that cannot resolve the differential diagnosis between entities showing overlapping features, like *SF3B1*-mutated MPNs and MDS-RS-T, for example. In fact, both entities share *SF3B1* and *JAK2* mutations as the dominant and often only molecular features together with a similar clinical presentation, especially in the early stages of disease. Only morphologic exclusion of dysplasia represents a reliable tool, at this time at least, to distinguish between the 2 entities.

Our study has a few limitations. The lack of a long follow-up did not allow us to draw any conclusions regarding patient outcome, including overall survival. MPNs have a long natural history, but NGS-based mutational analysis has become part of the routine workup of MPN patients only recently at our institutions, precluding the ability to reliably evaluate any direct prognostic impact of *SF3B1* mutations on MPN patients' outcome at this time. We chose to limit this study to cases of MPNs encountered in chronic phase and excluded cases in accelerated and blastic phase, which may introduce a selection bias for patients who had not progressed over the variable follow-up periods between the initial diagnosis and the time point included in the study. Moreover, because the majority of the patients with *SF3B1*-mutations had been previously diagnosed and some had received therapies, this could confound comparison between the *SF3B1* wild-type cases, which were mostly new diagnoses. Nevertheless, the results of our study suggest that acquisition of *SF3B1* mutations in MPN is not associated with a myelodysplastic evolution.

5. Conclusions

In conclusion, *SF3B1* mutations were observed in 10% of MPN diagnosed at 2 large academic medical centers and were more commonly seen in cases with advanced fibrosis, both primary (PMF and MPN-U with fibrosis) and secondary (post-ET MF and post-PV MF). Although we cannot exclude the possibility of referral bias, our findings suggest that *SF3B1* mutation is not an infrequent finding in MPN, particularly PMF. *SF3B1*-mutated MPNs are morphologically, clinically, and molecularly indistinguishable from *SF3B1*-WT MPNs except for the presence of RS in a subset of cases. It is important for pathologists to be aware that a subset of otherwise classic MPN cases can show *SF3B1* mutations and RS either at presentation or during disease evolution. Mutations in *SF3B1* were accompanied by RS in a subset of cases but were not associated with a myelodysplastic progression. Careful morphological examination of the bone marrow and peripheral blood for morphologic dysplasia is crucial to reach the correct diagnosis and to avoid misdiagnosing these patients with MDS/MPN-RS-T.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.11.022.

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