Brief report

Characteristics and clinical correlates of *MPL* 515W>L/K mutation in essential thrombocythemia

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Among 994 patients with essential thrombocythemia (ET) who were genotyped for the *MPL*W515L/K mutation, 30 patients carrying the mutation were identified (3.0%), 8 of whom also displayed the *JAK2*V671F mutation. *MPL*W515L/K patients presented lower hemoglobin levels and higher platelet counts than did wild type (wt) *MPL*; these differences were highly significant compared with *MPL*wt/*JAK2*V617F–positive patients. Reduced hemoglobin and increased platelet levels were preferentially associated with the W515L and W515K alleles, respectively. *MPL* mutation was a significant risk factor for microvessel disturbances, suggesting platelet hyperreactivity associated with constitutively active MPL; arterial thromboses were increased only in comparison to *MPL*wt/*JAK2*wt patients. *MPL*W515L/K patients presented reduced total and erythroid bone marrow cellularity, whereas the numbers of megakaryocytes, megakaryocytic clusters, and small-sized megakaryocytes were all significantly increased. These data indicate that *MPL*W515L/K mutations do not define a distinct phenotype in ET, although some differences depended on the *JAK2*V617F mutational status of the counterpart. (Blood. 2008;112:844-847)

Introduction

Ninety-five percent of polycythemia vera[1-5] and approximately 60% of essential thrombocythemia (ET)[6-9] or primary myelofibrosis (PMF) patients[10-13] harbor a *JAK2*V617F mutation. Another recurrent molecular abnormality, involving a W to L or K transversion at *MPL* codon 515, has been reported in 5% of PMF and 1% of ET patients, sometimes coexisting with *JAK2*V617F.[14-17] The W>L allele conferred factor-independent growth to Ba/F3 cells and induced a disorder recapitulating human myelofibrosis in a murine bone marrow transplant model.[17]

Patients with myelofibrosis harboring *MPL*W515L/K mutations presented more severe anemia compared with *MPL* wild-type (wt), but no other meaningful characteristic was ascertained[16]; on the other hand, only a few ET patients with *MPL*W515L/K mutation have been reported to date.[14,17] The aim of this study was to describe the prevalence, characteristics, and clinical and laboratory features associated with *MPL*W515L/K mutation in a large population of ET patients.

Methods

This multicenter study was conducted within the Gruppo Italiano per le Malattie Ematologiche dell'Adulto (GIMEMA) myeloproliferative disease (MPD) Working Party; 6 centers reported an unselected population of 994 patients, for whom hematologic and clinical data at diagnosis and during follow-up (median follow-up, 59 months) were available. The study received formal institutional review board approval at the Coordinating Center in Florence (#2007/0047/08). Informed consent was obtained in accordance with the Declaration of Helsinki. Original diagnosis of ET was according to World Health Organization (WHO)[18] or polycythemia vera study group (PVSG)[19] criteria in 680 (68%) and 314 (32%) patients, respectively; in all of the latter, appropriateness of ET diagnosis according to WHO criteria was confirmed by ad-hoc reevaluation of bone marrow (BM) biopsies.

Splenomegaly was defined as a palpable organ below the left costal margin. Major thromboses at diagnosis or in the 2 preceding years[20] were recorded if objectively documented[21]; diagnostic criteria for thrombosis and major bleeding have been defined elsewhere.[5] Microvessel disturbances (headache, acral paresthesia, erythromelalgia, transient neurologic and visual disturbances) were considered only if they were described from the patient as nonoccasional, of recent onset, and often ameliorated by aspirin; superficial venous thrombosis/thrombophlebitis was ruled out in case of erythromelalgia. Pruritus was recorded when it was described as diffuse, nonoccasional, itching exacerbated by water contact. Diagnosis of evolution to post-ET myelofibrosis fulfilled recent criteria.[22]

The *JAK2*V617F allele burden was measured by real time quantitative–polymerase chain reaction (RTQ-PCR) in granulocytic DNA.[23,24] Genotyping for *MPL*W515L/K was performed using a novel RTQ-PCR assay, based on locked nucleic acid probes, which reliably detected less than or equal to 0.1% mutant allele in a wild-type background[25]; the procedure is described

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Table 1. Laboratory and clinical characteristics of *MPL* mutant patients compared with *MPL* wild-type patients who were categorized according to their *JAK2*V617F mutational status

		MPL wild-type		*P*		
	***MPL*, mutant (A)**	***JAK2*, wild-type (B)**	***JAK2*V617F, mutant (C)**	**(A) vs (B)**	**(A) vs (C)**	**(B) vs (C)**
Number of patients	30	418	546	—	—	—
Male, no (%)	9 (30%)	144 (34%)	170 (31%)	.7	.9	.3
Median age, y (range)	56 (22-84)	50 (8-87)	56 (15-97)	**.006**	.33	**<.001**
MPL mutant allele burden, %*	39 ± 27	—	—	—	—	—
*JAK2*V617F allele burden, %*	11 ± 8†	—	25 ± 13	—	**.002**	—
White blood cell count, $\times 10^9$/L*	8.8 ± 3.1	8.5 ± 2.1	9.5 ± 3.1	.8	.2	**<.001**
Hemoglobin, g/L*	134 ± 13	135 ± 22	142 ± 21	.7	**<.001**	**<.001**
Platelet count, $\times 10^9$/L*	956 ± 331	935 ± 315	791 ± 211	.8	**.004**	**<.001**
LDH, U/L*	459 ± 182	336 ± 128	338 ± 116	**.03**	**.005**	.1
Serum ferritin, μg/L*	110 ± 82	82 ± 85	93 ± 96	.08	.1	.7
Splenomegaly, no (%; n = 892)‡	5 (17%)	71 (20%)	107 (21%)	.9	.9	.8
Pruritus, no (%; n = 663)‡	4 (13%)	13 (5%)	34 (10%)	.1	.5	.2
Arterial events at diagnosis, no (%)	3 (10%)	24 (6%)	66 (12%)	.4	.9	**.008**
Arterial events at follow-up, no (%)	4 (13%)	6 (1%)	20 (4%)	**.002**	.05	.04
Venous events at diagnosis, no (%)	1 (3%)	10 (2%)	29 (5%)	.5	.9	.09
Venous events at follow-up, no (%)	2 (7%)	5 (1%)	12 (2%)	.07	.1	.3
Microvessel disturbances, no (%)	18 (60%)	106 (25%)	170 (31%)	**<.001**	**.001**	.1
Major hemorrhage, no (%)§	2 (7%)	15 (4%)	20 (4%)	.1	.1	.8

Unless otherwise specified, all data were collected at diagnosis. *P* values in bold indicate statistically significant differences ($P < .05$).

*Mean value (± SD) is reported.

†Refers only to the 8 patients who harbored both *JAK2*V617F and *MPL*W515L/K mutation.

‡Numbers within parentheses indicate the number of patients for whom information was available.

§Includes patients with hemorrhage either at diagnosis, during follow-up, or both.

in Document S1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

BM biopsies from *MPL*W515L/K and *MPL*wt patients were analyzed blind by 3 hemopathologists. In addition, digital pictures were taken for to measure cellularity, fibrosis, number of megakaryocytes, small megakaryocytes, and megakaryocytic clusters (as described in Document S1).[26,27]

Statistical analysis was performed with GraphPad InStat (San Diego, CA) and SPSS (Chicago, IL) software. We used the chi-square or Fisher exact test (2×2 table) to compare categorical variables, the Mann-Whitney U test for continuous variables, and unconditional logistic regression models with backward stepwise where appropriate. Significance level was *P* less than .05 in 2-sided tests.

Results and discussion

Characteristics of the 994 ET patients, categorized according to their genotype, are shown in Table 1. We found 30 patients (3.0%) harboring *MPL*W515L/K mutation, of whom 18 (60%) had the W515L and 12 (40%) the W515K allele. *MPL* mutant allele burden greater than 50% was found in 50% of W515K patients compared with 17% of W515L patients ($P = .04$). *JAK2*V617F mutation was harbored by 55.7% of the patients; 25 of them (4.5%) had greater than 50% V617F allele. *MPL*W515 and *JAK2*V617F mutation coexisted in 3 patients with W515L and 5 with W5151K allele, while no patient contemporarily harbored both *MPL* mutant alleles. The median V617F allele burden in double *MPL*W515L/K/*JAK2*V617F mutant was significantly lower than in *MPL*wt/*JAK2*V617F patients (8% vs 26%, $P = .002$), and for the purposes of this study *MPL*W515L/K patients were considered irrespective of their *JAK2* genotype. Prevalence of *MPL*W515L/K mutation was 3.3%, 3.8%, and 2.5% in samples genotyped within 1 year from diagnosis (30%), within 3 years (21%) or later (49%), respectively, suggesting that the mutation is present at diagnosis rather than being acquired during the course of the disease.

Compared with *MPL*wt/*JAK2*wt, *MPL*W515L/K patients were older, but did not present any difference in hematologic parameters (Table 1); however, hemoglobin level was significantly lower and platelet count significantly higher compared with *MPL*wt/*JAK2*V617F patients. Arterial thrombosis during follow-up occurred at significantly higher rate in *MPL*W515L/K patients than in *MPL*wt/*JAK2*wt, while conversely more arterial events at diagnosis were found in *MPL*wt/*JAK2*V617F than in *MPL*wt/*JAK2*wt; the low number of events precluded multivariate analysis, and no definite statement in this regard could be made. There was no difference in venous thromboses or hemorrhages between *MPL* mutant and *MPL*wt patients. However, microvessel disturbances were significantly more frequent in *MPL*W515L/K than in *MPL*wt patients, and both *JAK2*wt and *JAK2*V617F; in multivariate analysis, the relative risk was 2.0 (95% confidence interval, 1.5-2.7; $P < .001$) using *MPL*wt patients as reference. *MPL*W515L/K did not impact on transformation to myelofibrosis that occurred in 6.4% of *MPL*W515L/K compared with 3.2% and 5.0% of *MPL*wt/*JAK2*wt and *MPL*wt/*JAK2*V617F patients, respectively. Finally, analysis of BM biopsies revealed significantly reduced total and erythroid cellularity in *MPL*W515L/K patients, associated with increased number of megakaryocytes, megakaryocytic clusters and small megakaryocytes, but no increase in reticulin fibrosis (Table S1).

*MPL*W515L and W515K mutant patients were then considered separately to ascertain characteristics associated with each mutant allele (Table 2). W515L mutant patients were older than W515K and *MPL*wt/*JAK2*wt patients, and displayed significantly lower hemoglobin level compared with W515K and *MPL*wt patients, particularly if they harbored *JAK2*V617F mutation. Conversely, platelet count was significantly higher in W515K patients than in W515L and *MPL*wt/*JAK2*V617F patients. There was no difference between W515L and W515K patients in splenomegaly, pruritus, hemorrhage, arterial or venous thrombosis or microvessel disturbances that occurred in 55% and 66% of patients, respectively.

In this series of ET patients we found an association of *MPL*W515L/K mutation with lower hemoglobin level, as also

Table 2. Laboratory and clinical characteristics of *MPL*W515L and W5151K mutated patients compared with *MPL* wild-type patients, who were categorized according to their *JAK2*V617F mutational status

	MPL		*JAK2*		*P value*				
	W515L (A)	W515K (B)	wild-type (C)	V617F (D)	(A) vs (B)	(A) vs (C)	(A) vs (D)	(B) vs (C)	(B) vs (D)
Number of patients	18	12	418	546	—	—	—	—	—
Male, no (%)	7 (38.8)	2 (16.6)	144 (34)	170 (31)	.2	.7	.8	.5	.5
Median age, y*	67 (31-84)	48 (22-66)	50 (8-87)	56 (15-97)	**.004**	**.01**	.89	.6	.1
MPL mutant allele burden, %†	37 ± 23	53 ± 26	—	—	.09	—	—	—	—
JAK2V617F mutated, no (%)	3 (17%)	5 (42%)	—	—	.2	—	—	—	—
JAK2V617F allele burden, %†	13 ± 14	10 ± 15	—	25 ± 13	.9	—	**.02**	—	**.01**
White blood cell count, × 10^9/L†	8.3 ± 2.9	9.5 ± 3.3	8.5 ± 2.1	9.5 ± 3.1	.5	.6	.1	.4	.9
Hemoglobin, g/L†	129 ± 13	142 ± 90	135 ± 22	142 ± 21	**.003**	**.01**	**<.001**	.07	.6
Platelet count, × 10^9/L†	882 ± 358	1060 ± 270	935 ± 315	791 ± 211	**.01**	.2	.1	.07	**<.001**
LDH, U/L†	493 ± 249	431 ± 102	336 ± 128	338 ± 116	.8	.1	**.01**	.06	**.02**
Serum ferritin, μg/L†	140 ± 106	80 ± 34	82 ± 85	93 ± 96	.6	.07	.23	.5	.6

All data were collected at diagnosis. *P* values in bold indicate statistically significant differences ($P < .05$).
*Median values (range) are shown.
†Mean values (± SD) are shown.

reported in myelofibrosis,[16] and with higher platelet count, especially in comparison to *MPL*wt/*JAK2*V617F counterpart. These abnormalities mimic the phenotype of mice transplanted with *MPL*W515L-transduced cells that presented thrombocytosis and a shift of erythroid cells toward more immature phenotype[17]; furthermore, *MPL*W515L/K progenitor cells from PMF patients were found to generate thrombopoietin-independent megakaryocytic colonies but no erythropoietin-independent erythroid colonies, and overall erythroid progenitor cloning efficiency was reduced.[28,29] In keeping with this was the reduced erythroid cellularity with increased megakaryocytes we documented in BM biopsies from *MPL*W515L/K patients; this would support a preferential expansion of megakaryocytic lineage at the expense of erythroid differentiation due to activating *MP*L mutation.[30]

A novel finding was the association of *MPL*W515L/K with microvessel disturbances; because activation of Mpl by thrombopoietin enhances normal platelet function,[31] and abnormal activation of ET platelets by thrombopoietin preincubation has been described,[32] we hypothesize that platelets from *MPL*W515L/K patients present constitutively enhanced reactivity.

In summary, this large survey indicated that prevalence of *MPL*W515L/K mutation in ET is higher than originally described and closer to myelofibrosis,[14,16,17] as also observed in the PT-1 cohort[33]; however, because *MPL* mutant alleles other than W515L/K have also been described,[34] overall prevalence of *MPL* mutation in ET might be slightly underestimated. Although our findings did not result in the definition of a discrete clinical phenotype, they underscore the relevance of *MPL* mutational screening in the diagnostic work-up of suspected ET as a tool to establish the occurrence of a clonal myeloproliferation[35] in cases that lack the *JAK2*V617F mutation.

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Authorship

Contribution: A.M.V. designed research, analyzed data, and wrote the manuscript; E. Antonioli collected and analyzed clinical data, and helped write the manuscript; P.G. collected and analyzed clinical data, assayed *JAK2* and *MPL* mutations, reviewed biopsies, and helped write the manuscript; A.P. performed *MPL* mutational assay and helped biopsy data collection; V.G., G.B., M.R., G.S., F.L.C., F.D., L.V., S.F., E. Ammatuna, A.R., L.V., and A.B. collected clinical data and biologic samples; V.C., R.A., and S.D.L. reviewed biopsies; G.C. processed bone marrow biopsies; G.B., F.L.C., and T.B. discussed data and helped write the manuscript. All authors reviewed and gave final approval to the manuscript.

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