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ORIGINAL ARTICLE

IDH1 and *IDH2* mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis

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In a multi-institutional collaborative project, 1473 patients with myeloproliferative neoplasms (MPN) were screened for isocitrate dehydrogenase 1 (*IDH1*)/*IDH2* mutations: 594 essential thrombocythemia (ET), 421 polycythemia vera (PV), 312 primary myelofibrosis (PMF), 95 post-PV/ET MF and 51 blast-phase MPN. A total of 38 *IDH* mutations (18 *IDH1*-R132, 19 *IDH2*-R140 and 1 *IDH2*-R172) were detected: 5 (0.8%) ET, 8 (1.9%) PV, 13 (4.2%) PMF, 1 (1%) post-PV/ET MF and 11 (21.6%) blast-phase MPN ($P<0.01$). Mutant *IDH* was documented in the presence or absence of *JAK2*, *MPL* and *TET2* mutations, with similar mutational frequencies. However, *IDH*-mutated patients were more likely to be nullizygous for *JAK2* 46/1 haplotype, especially in PMF ($P=0.04$), and less likely to display complex karyotype, in blast-phase disease ($P<0.01$). In chronic-phase PMF, *JAK2* 46/1 haplotype nullizygosity ($P<0.01$; hazard ratio (HR) 2.9, 95% confidence interval (CI) 1.7–5.2), but not *IDH* mutational status ($P=0.55$; HR 1.3, 95% CI 0.5–3.4), had an adverse effect on survival. This was confirmed by multivariable analysis. In contrast, in both blast-phase PMF ($P=0.04$) and blast-phase MPN ($P=0.01$), the presence of an *IDH* mutation predicted worse survival. The current study clarifies disease- and stage-specific *IDH* mutation incidence and prognostic relevance in MPN and provides additional evidence for the biological effect of distinct *JAK2* haplotypes.
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Introduction

Despite the seminal discovery of *JAK2* or *MPL* mutations in the majority of patients with *BCR-ABL1*-negative myeloproliferative neoplasms (MPN),[1–4] it is becoming increasingly evident that these mutations do not signify either disease-initiating or leukemia-promoting events.[5,6] It is therefore important to keep looking for additional molecular alterations to clarify the genetic underpinnings of both chronic- and blast-phase MPN. In the last 2 years, mutations involving *TET2*, *ASXL1* and *CBL* have been described in some patients with *BCR-ABL1*-negative MPN, including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).[7] The precise pathogenetic contribution of these mutations and their clinical relevance are currently under investigation. The glioma-associated[8] isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* mutations are the latest to be added to the 'MPN mutations list'.[9]

IDH1, located on chromosome 2q33.3, and *IDH2*, located on chromosome 15q26.1, encode enzymes that catalyze oxidative decarboxylation of isocitrate to α-ketoglutarate. IDH1 (cytoplasm and peroxisome) and IDH2 (mitochondria) use $NADP^+$ as a co-factor to generate NADPH, which is important in the production of intracellular glutathione. Intact IDH activity is therefore necessary for cellular protection from oxidative stress. Mutant IDH has decreased affinity to isocitrate, but displays neomorphic catalytic activity toward α-ketoglutarate, the net result being decreased supply of α-ketoglutarate and accumulation of 2-hydroxyglutarate.[10–13] It is currently believed that these intracellular changes facilitate oncogenic pathways including activation of HIF-1α.[10]

IDH1 and *IDH2* mutations were first described in low-grade gliomas/secondary glioblastomas[8] and subsequently in acute myeloid leukemia (AML),[14] with respective mutational frequencies of ~70 and 8%. We recently screened 200 patients with either chronic- or blast-phase MPN for *IDH* mutations, and identified 9 patients with either *IDH1* ($n=5$) or *IDH2* ($n=4$) mutations.[9] Mutational frequencies were ~21% for blast-phase MPN and ~4% for PMF. In the current study, we expanded our study cohort to include 1473 patients recruited from three MPN centers of excellence, with the intent to accurately describe the prevalence of *IDH* mutations in chronic-, fibrotic- and blast-phase PV, ET and PMF. In addition, *IDH*-mutated patients were analyzed for their cytogenetic and molecular (that is, *JAK2*, *MPL* and *TET2* mutation and *JAK2* haplotype status) phenotype, as well as their prognostic relevance.

Materials and methods

This study was approved by the Mayo Clinic institutional review board. All patients provided authorization for use of their medical records for research purposes, and the research was carried out according to the principles of the Declaration of Helsinki. Patient samples were obtained from the Mayo Clinic, Harvard Medical Institute and University of Florence. Mutational analyses were performed on DNA derived from either bone marrow or peripheral blood granulocytes. *JAK2* 46/1 haplotype analysis on patient samples accrued from Harvard was performed on germline DNA. Diagnoses of MPN, post-PV/ET MF

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and AML, in patient samples accrued from the Mayo Clinic and the University of Florence, were according to the World Health Organization and International Working Group criteria.[7,15] Diagnoses in patients accrued from Harvard were self-reported during an internet-based collection of samples, as previously detailed.[16]

DNA from either bone marrow (Mayo Clinic samples) or granulocytes (samples from Harvard and the University of Florence) was extracted using conventional methods. *MPL*, *JAK2* and *TET2* mutation and *JAK2* haplotype analyses were performed according to previously published methods.[4,17–19] With regard to *IDH* mutation analysis, Harvard patient samples were analyzed using the following primers for *IDH1*, which cover amino acid residues 41–138: sense, 5′-TGTGTTGAGAT GGACGCCTA-3′ and anti-sense, 5′-GGTGTACTCAGAGCCTTC GC-3′. Sequencing of IDH2 used primers that covered amino acid residues 125–226: sense, 5′-CTGCCTCTTTGTGGCCTA AG-3′ and anti-sense, 5′-ATTCTGGTTGAAAGATGGCG-3′. Sequence analysis was performed using Mutation Surveyor (SoftGenetics, State College, PA, USA) and all mutations were validated by repeat PCR and sequencing on unamplified DNA from the archival sample.

Mayo Clinic and University of Florence patient samples were screened for *IDH1* and *IDH2* mutations by direct sequencing

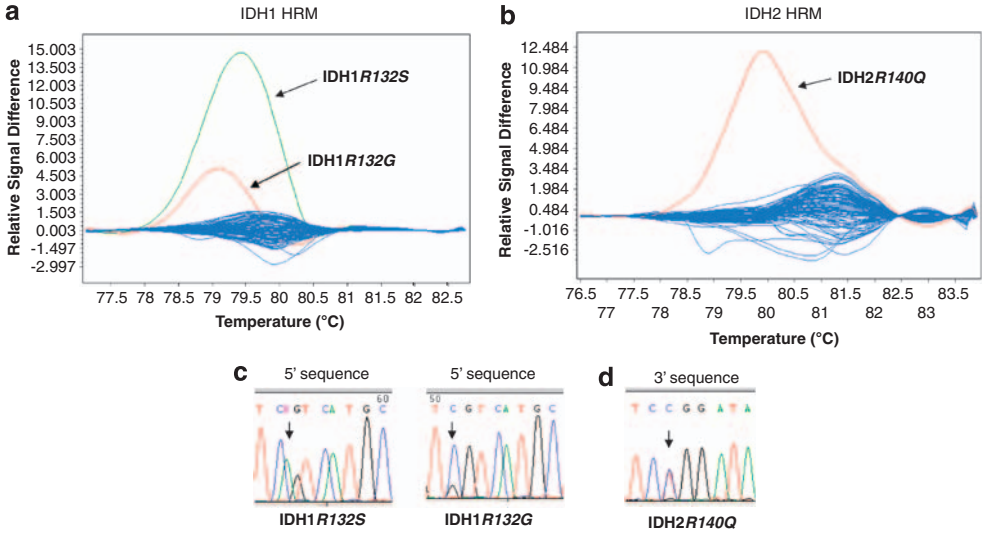

Figure 1 High-resolution melting (HRM) normalized and temperature-shifted difference plot for *IDH1* (**a**) and *IDH2* (**b**) and corresponding sequences (**c** and **d**).

Table 1 Specific diagnoses, age/sex distribution, *JAK2*, *MPL* and *TET2* mutational status and *JAK2* non-46/1 haplotype frequency in 1473 patients with polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), post-PV MF, post-ET MF, post-PV acute myeloid leukemia (post-PV AML), post-ET AML or post-PMF AML

MPN center	*Diagnosis*	N	*Median age in years (range)*	*Males (%)*	*JAK2 mutation frequency*	*MPL mutation frequency*	*TET2 mutation frequency*	*JAK2 46/1 nullizygous frequency*
Florence	PV	150	62 (16–91)	66	83% (123/149)[a]	NA	NA	6% (1/18)
(*n* = 522)	ET	199	56 (13–93)	37	63% (124/198)	2.2% (4/184)	NA	52% (25/48)
	PMF	107	63 (16–90)	67	65% (69/106)	4% (4/98)	NA	39% (37/96)
	Post-PV MF	32	62 (48–78)	47	100% (32/32)	0% (0/28)	NA	0% (0/16)
	Post-ET MF	26	63 (33–82)	50	39% (10/26)	13% (3/24)	NA	27% (3/11)
	Post-PV AML	1	66	0	100% (1/1)	0% (0/1)	NA	NA
	Post-ET AML	2	65–70	0	50% (1/2)	0% (0/1)	NA	NA
	Post-PMF AML	5	73 (67–83)	80	20% (1/5)	0% (0/5)	NA	20% (1/5)
Harvard	PV	159	59 (32–85)	48	93% (139/150)[a]	0% (0/159)	9.4% (15/159)	23% (29/125)
(*n* = 322)	ET	124	57 (31–84)	26	31% (35/114)	3.2% (4/124)	8% (10/124)	42% (41/98)
	PMF	39	64 (50–70)	49	42% (16/38)	5.1% (2/39)	7.7% (3/39)	22% (5/23)
Mayo	PV	112	66 (21–95)	48	95% (106/112)[a]	1.8% (1/56)	15.7% (14/89)	25% (25/99)
(*n* = 629)	ET	271	63 (15–87)	38	49% (132/271)	4.9% (7/143)	5.7% (3/53)	34% (91/266)
	PMF	166	62 (35–82)	67	57% (95/166)	10% (11/108)	18% (10/57)	35% (55/158)
	Post-PV MF	22	65 (47–75)	64	100% (22/22)	7.7% (1/13)	7.7% (1/13)	5% (1/20)
	Post-ET MF	15	63 (39–75)	80	47% (7/15)	10% (1/10)	12.5% (1/8)	31% (4/13)
	Post-PV AML	11	64 (48–87)	64	100% (11/11)	0% (0/7)	20% (1/5)	36% (4/11)
	Post-ET AML	5	64 (50–75)	60	60% (3/5)	0% (0/5)	25% (1/4)	20% (1/5)
	Post-PMF AML	27	66 (49–83)	74	48% (13/27)	9% (2/22)	0% (0/7)	35% (8/23)

Abbreviation: NA, not done or not available.
[a]Includes *JAK2* exon 12 mutations: two cases from Mayo clinic and one case from Harvard.

and/or high-resolution melting assay. Direct sequencing for *IDH1* exon 4 mutations was carried out using the following primer sequences: sense, 5′-CGGTCTTCAGAGAAGCCATT-3′ and anti-sense, 5′-CACATTATTGCCAACATGAC-3′.[18] *IDH2* exon 4 was amplified using sense, 5′-CCACTATTATCTCTGTC CTC-3′ and anti-sense, 5′-GCTAGGCGAGGAGCTCCAGT-3′.[19] Both reactions were performed in 25 μl volume containing 100 ng of DNA, 0.25 U Taq polymerase, 0.3 mM each of dATP, dCTP, dGTP and dTTP, 5 μl of a 10× PCR buffer (Roche Diagnostics, Indianapolis, IN, USA) and 0.2 μM each of sense and anti-sense primers. The reaction was denatured at 94 °C for 3 min followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 57 °C for 30 s and extension at 72 °C for 40 s. After a final extension at 72 °C for 2 min, the products were confirmed by running on 1.3% agarose gel and purified using Qiagen's PCR Quick Purification Kit. The product was sequenced using the ABI PRISM 3730xl analyzer (Applied Biosystems Inc, Foster City, CA, USA) to screen for the presence of mutations.

High-resolution melting was performed using the LightCycler 480 real-time PCR system (Roche Diagnostics), using the above-mentioned primers for *IDH1* mutations (R130) and the following primers for *IDH2* mutations (R140 and R172): R140 sense, 5′-GCTGAAGAAGATGTGGAA-3′ and anti-sense, 5′-TGATGG GCTCCCGGAAGA-3′; R172 sense, 5′-CCAAGCCCATCACCAT TG-3′ and anti-sense, 5′-CCCAGGTCAGTGGATCCC-3′ (Figure 1).

Conventional statistical procedures were used (SAS Institute, Cary, NC, USA). All statistically analyzed data were obtained at time of *IDH* mutation analysis. All *P*-values were two-tailed and statistical significance was set at the level of $P<0.05$. Categorical variables were described as count and relative frequency and compared by χ^2 statistics. Comparison of continuous variables between categories was performed by the Mann–Whitney *U*-test. Survival analysis was performed by the Kaplan–Meier method taking the interval from the date of diagnosis, for chronic-phase disease, or from the date of leukemic transformation, for blast-phase disease, to death or last contact. The log-rank test was used to compare survival data. Cox regression model was used for multivariable analysis.

Results

Disease- and stage-specific IDH mutational frequencies

A total of 1473 patients with *BCR–ABL1*-negative MPN were recruited from the Mayo Clinic, Rochester, MN, USA ($n=629$), University of Florence, Florence, Italy ($n=522$) and Harvard Medical Institute, Boston, Massachusetts, USA ($n=322$). Specific diagnoses included ET ($n=594$), PV ($n=421$), PMF ($n=312$), post-PV MF ($n=54$), post-ET MF ($n=41$), post-PV AML ($n=12$), post-ET AML ($n=7$) and post-PMF AML ($n=32$). Table 1 provides clinical and laboratory details of the study population including age and sex distribution, specific diagnoses and *JAK2*, *MPL* and *TET2* mutational and *JAK2* 46/1 haplotype status, stratified by center of patient recruitment. A total of 38 *IDH* mutations were documented (Table 2): 18 involved *IDH1* (10 R132S, 7 R132C and 1 R132G) and 20 *IDH2* (18 R140Q, 1 R140W and 1 R172G). *IDH* mutations were infrequent in chronic- or fibrotic-phase disease and significantly more prevalent in blast-phase disease ($P<0.01$; Table 3): 5 (0.8%) in ET, 8 (1.9%) in PV, 13 (4.1%) in PMF, 1 (1%) in post-ET/PV MF, none in blast-phase ET, 3 (25%) in blast-phase PV and 8 (25%) in blast-phase PMF.

Correlation of IDH mutations with other MPN-associated mutations and JAK2 46/1 haplotype

Considering the preponderance of informative cases with centrally confirmed diagnosis and availability of a more complete laboratory data, the current analysis was limited to patients from the Mayo Clinic cohort ($n=629$). *IDH* mutational frequencies were similar among *JAK2*- (3.6%), *MPL*- (4.3%) and *TET2* (3.2%)-mutated patients and their respective mutation-negative counterparts (4.2, 5.3 and 6.3%; Table 3). In other words, mutant *IDH* was shown to co-occur with a *JAK2*, *MPL* or *TET2* mutation, and mutational frequency did not appear to be influenced by either the type of the coexisting mutation ($P=0.96$) or the presence or absence of each specific mutation (Table 3). However, *IDH*-mutated cases were more likely to be nullizygous for *JAK2* 46/1 haplotype, especially when analysis was restricted to informative (that is, with *JAK2* 46/1 haplotype information) patients with chronic- ($n=158$) or blast ($n=23$)-phase PMF, analyzed together ($P=0.007$) or separately ($P=0.04$; Table 4).

Clinical correlates and prognostic relevance

To avoid disease- or stage-specific confounding factors, as well as assure adequate sample size of informative cases, clinical correlative and prognostic studies were limited to PMF. In this patient cohort, detailed clinical information was available in 111 patients with chronic-phase PMF (including 7 *IDH*-mutated cases) and 27 patients with blast-phase PMF (including 8 *IDH*-mutated cases), both patient populations were accrued from the Mayo Clinic cohort. In both chronic- and blast-phase PMF, the presence of *IDH* mutations was not influenced by either age ($P=0.51$ and 0.70, respectively) or gender ($P=0.09$ and 0.3, respectively). In chronic-phase disease, comparison of prognostically relevant disease variables at diagnosis revealed that cytogenetic findings in *IDH*-mutated cases often belonged to a low- or intermediate-risk category,[20] although the difference was not statistically significant (Table 4). Similarly, *IDH*-mutated blast-phase PMF was less likely to display complex karyotype (0 vs 64% in *IDH*-unmutated cases; $P=0.001$).

In addition to biological implications, the aforementioned associations of *IDH* mutations with favorable cytogenetic profile and *JAK2* 46/1 haplotype nullizygosity, both which have previously been shown to be prognostically relevant,[19,20] mandated their inclusion as covariates during multivariable survival analysis. In chronic-phase PMF, univariate analysis showed statistically significant adverse survival effect from *JAK2* 46/1 haplotype nullizygosity ($P=0.0001$; 34 nullizygous vs 74 not nullizygous), high-risk karyotype ($P<0.0001$; 13 high-risk vs 98 not high-risk) and higher International Prognostic Scoring System (IPSS; 27 high, 29 intermediate-2, 30 intermediate-1 and 25 low-risk patients)[21] risk score ($P<0.0001$), but not from *IDH* mutational status ($P=0.54$; 7 mutated vs 104 unmutated; Figure 2). Multivariable analysis confirmed the independent prognostic value of *JAK2* 46/1 haplotype status (hazard ratio (HR) 2.2, 95% confidence interval (CI) 1.2–4.2), karyotype (HR 2.8, 95% CI 1.3–5.9) and IPSS risk score (HR 4.8, 95% CI 2.0–11.5).

In blast-phase PMF, despite its association with noncomplex karyotype, the presence of mutant *IDH* predicted shortened survival, calculated from the time of disease transformation ($P=0.04$), and there was a similar trend for *JAK2* non-46/1 haplotype ($P=0.14$; Figure 3). Significance was lost for both during multivariable analysis, probably because of small sample size. *IDH* mutation status also predicted worse survival when the analysis included all blast-phase MPN cases from the Mayo

Table 2 Clinical, cytogenetic and molecular details, at time of mutation analysis, of 38 *IDH*-mutated patients with chronic- or advanced-phase polycythemia vera, essential thrombocythemia or primary myelofibrosis

	Specific diagnosis	*Age (years) and sex*	*IDH mutation variant*	*JAK2 V617F burden*	*MPL mutation status*	*TET2 mutation status*	*JAK2 haplotype status*	*Karyotype*	*Antecedent MPN diagnosis to IDH analysis*	*IDH analysis to last f/u*	*Status at last f/u*
1	ET (Mayo)	26/F	IDH2 R140Q	0%	WT	WT	Heterozygous	NN	0 month	6 years	Alive with ET
2	ET (Mayo)	38/F	IDH2 R140Q	5%	WT	WT	Heterozygous	NA	5 years	NA	Lost to follow-up
3	ET (Florence)	80/F	IDH2 R140Q	73%	WT	NA	NA	NA	1.1 years	4 months	Alive with ET
4	ET (Florence)	79/M	IDH2 R140Q	64%	WT	NA	Nullizygous	NA	7 months	2 years	Alive with ET
5	ET (Florence)	65/F	IDH1 R132C	0%	NA	NA	NA	NA	7.2 years	1 month	Alive with ET
6	PV (Harvard)	52/F	IDH2 R140Q	58%	WT	WT	Heterozygous	NN	NA	NA	NA
7	PV (Harvard)	47/M	IDH2 R140Q	90%	WT	WT	Homozygous	NN	NA	NA	NA
8	PV (Florence)	79/F	IDH1 R132S	50%	NA	ND	NA	NA	0 month	0 month	Alive with PV
9	PV (Florence)	49/M	IDH2 R140Q	25%	NA	ND	NA	NA	1 month	0 month	Alive with PV
10	PV (Mayo)	82/M	IDH1 R132C	1%	WT	WT	Heterozygous	NN	4 years	5 months	Dead with AML
11	PV (Mayo)	50/M	IDH2 R140Q	25%	WT	WT	NA	NN	4 months	5 years	Alive with PV
12	PV (Mayo)	75/M	IDH2 R140Q	11%	WT	WT	Heterozygous	NN	0 month	1 month	Alive with PV
13	PV (Mayo)	82/F	IDH2 R140Q	51%	WT	WT	Homozygous	NN	29 months	3.4 years	Dead with PV
14	PMF (Harvard)	76/M	IDH2 R140Q	72%	WT	WT	Homozygous	NN	NA	NA	NA
15	PMF (Florence)	57/M	IDH1 R132S	65%	WT	ND	Nullizygous	NA	0 month	6 years	Alive with PMF
16	PMF (Florence)	80/M	IDH1 R132S	50%	WT	ND	Nullizygous	NA	0 month	1 year	Alive with PMF
17	PMF (Florence)	62/M	IDH1 R132G	10%	WT	ND	NA	NA	2 months	4 months	Alive with PMF
18	PMF (Florence)	72/M	IDH2 R140Q	70%	WT	NA	Heterozygous	NA	0 month	1.8 years	Alive with PMF
19	PMF (Florence)	50/M	IDH1 R132S	54%	WT	NA	Heterozygous	NA	0 month	5.2 years	Alive with PMF
20	PMF (Mayo)	74/M	IDH1 R132S	0%	WT	Mutated	Nullizygous	NN	0 month	2.8 years	Dead with PMF
21	PMF (Mayo)	69/M	IDH2 R140Q	22%	WT	NA	Nullizygous	NN	4 months	11 months	Dead with AML
22	PMF (Mayo)	73/M	IDH1 R132S	26%	WT	NA	Nullizygous	NN	1 month	6 months	Dead from unknown cause
23	PMF (Mayo)	69/M	IDH2 R140Q	0%	WT	WT	Nullizygous	NN	2 months	3 months	Dead from unknown cause
24	PMF (Mayo)	58/F	IDH1 R132C	0%	WT	NA	Nullizygous	NN	3 years	3.2 years	Dead with AML
25	PMF (Mayo)	53/M	IDH2 R140Q	0%	WT	WT	Heterozygous	NN	0 month	6 years	Alive with PMF
26	PMF (Mayo)	50/F	IDH2 R172G	0%	WT	WT	Heterozygous	+9	1 year	5 years	Alive with PMF
27	Post-PV MF (Florence)	56/F	IDH2 R140Q	84%	WT	NA	Homozygous	NA	1 year	1.4 years	Dead with AML
28	Post-PMF AML (Mayo)	62/M	IDH2 R140W	65%	WT	NA	Nullizygous	+21	8 months	2 months	Dead with AML
29	Post-PMF AML (Mayo)	64/M	IDH1 R132C	0%	WT	WT	Heterozygous	15q−	5.8 years	4 months	Dead with AML
30	Post-PMF AML (Mayo)	73/M	IDH1 R132C	0%	WT	WT	Nullizygous	NN	2 years	2.5 months	Dead with AML
31	Post-PMF AML (Mayo)	61/M	IDH2 R140Q	0%	WT	NA	Nullizygous	−7,20q−	5 months	5 months	Dead with AML
32	Post-PMF AML (Mayo)	66/M	IDH1 R132S	96%	WT	WT	Homozygous	+2	4.5 years	1 month	Dead with AML
33	Post-PMF AML (Mayo)	82/F	IDH1 R132S	1%	WT	NA	Nullizygous	+9	2 months	<1 month	Dead with AML
34	Post-PMF AML (Mayo)	80/M	IDH1 R132S	1%	WT	NA	Homozygous	NA	2.2 years	1 month	Dead with AML
35	Post-PMF AML (Mayo)	81/M	IDH1 R132C	0%	MPLW515L	WT	Nullizygous	T(8;21)	2 years	2 months	Dead with AML
36	Post-PV AML (Mayo)	82/M	IDH1 R132C	7%	WT	NA	Heterozygous	5q−	2.8 years	3 months	Dead with AML
37	Post-PV AML (Mayo)	67/M	IDH2 R140Q	3%	WT	NA	Heterozygous	−5 and [a]	NA	1.5 months	Dead with AML
38	Post-PV AML (Mayo)	53/M	IDH1 R132S	80%	WT	NA	Nullizygous	der(1;7),+8	16 years	8 months	Dead with AML

Abbreviations: AML, acute myeloid leukemia; ET, essential thrombocythemia; IDH, isocitrate dehydrogenase; MPN, myeloproliferative neoplasm; NA, information not available; ND, not done; NN, normal cytogenetics; PMF, primary myelofibrosis; PV, polycythemia vera; WT, wild type.
[a]Multiple trisomies: +2, +3, +6, +8, +10, +11, +12, +13, +14, +19, +20, +21.

Table 3 *IDH* mutational frequencies in 1473 patients with polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), post-PV MF, post-ET MF, post-PV acute myeloid leukemia (post-PV AML), post-ET AML or post-PMF AML

Variables	*Number of patients*	*IDH mutated (IDH1 or IDH2), n (%)*	*IDH1 mutated, n*	*IDH2 mutated, n*	P-*value*
All patients	1473	38	18	20	<0.01
PV	421	8 (1.9%)	2	6	
ET	594	5 (0.8%)	1	4	
PMF	312	13 (4.2%)	7	6	
Post-PV MF	54	1 (1.9%)	0	1	
Post-ET MF	41	0	0	0	
Post-PV AML	12	3 (25%)	2	1	
Post-ET AML	7	0	0	0	
Post-PMF AML	32	8 (25%)	6	2	
JAK2 mutated vs wild type ($n=629$)[a]	389 vs 240	14 (3.6%) vs 10 (4.2%)			0.72
MPL mutated vs wild type ($n=364$)[a]	23 vs 341	1 (4.3%) vs 18 (5.3%)			0.85
TET2 mutated vs wild type ($n=237$)[a]	31 vs 206	1 (3.2%) vs 13 (6.3%)			0.5
JAK2 46/1 nullizygous vs not nullizygous ($n=596$)[a]	189 vs 407	11 (5.8%) vs 12 (2.9%)			0.09

Abbreviations: AML, acute myeloid leukemia; IDH, isocitrate dehydrogenase; PMF, primary myelofibrosis.
[a]Analysis limited to Mayo clinic patients only and '*n*' signifies number of patients evaluated.

Table 4 *IDH* mutational frequencies in 193 Mayo clinic patients with chronic-phase ($n=166$) or blast-phase ($n=27$) primary myelofibrosis (PMF) stratified by *JAK2* mutational, *JAK2* 46/1 haplotype or cytogenetic status

Variables	N	*IDH mutated (IDH1 or IDH2), n (%)*	P-*value*
Chronic-phase PMF (JAK2V617F mutated vs wild type)	166 (95 vs 71)	7 (4.2%) (2 (2.1%) vs 5 (7%))	0.12
Blast-phase PMF (JAK2V617F mutated vs wild type)	27 (13 vs 14)	8 (30%) (4 (31%) vs 4 (29%))	0.9
Chronic-phase PMF (JAK2 46/1 nullizygous vs not nullizygous)	158 (55 vs 103)	7 (4.4%) (5 (9%) vs 2 (1.9%))	0.04
Blast-phase PMF (JAK2 46/1 nullizygous vs not nullizygous)	23 (8 vs 15)	8 (35%) (5 (63%) vs 3 (20%))	0.04
Chronic-phase PMF karyotype at diagnosis (high-risk karyotype vs not high-risk)	111 (13 vs 98)	7 (6.3%) (0 (0%) vs 7 (7.1%))	0.32
Blast-phase PMF karyotype at transformation (complex karyotype vs not complex)	22 (11 vs 11)	7 (32%) (0 vs 7 (64%))	0.001

Abbreviations: IDH, isocitrate dehydrogenase; N, number of patients evaluable; PMF, primary myelofibrosis.

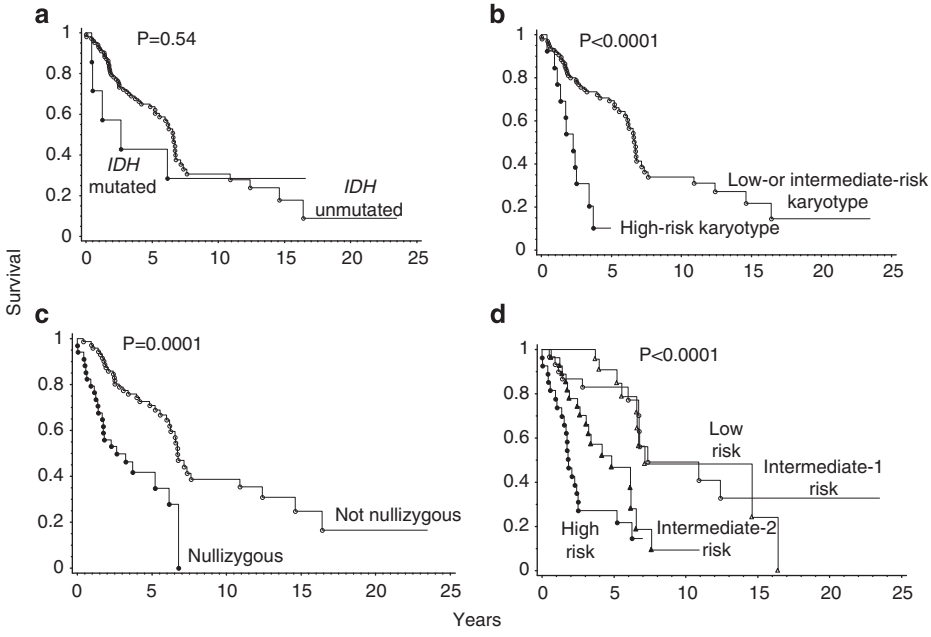

Figure 2 Survival curves of 111 patients with chronic-phase primary myelofibrosis stratified by their isocitrate dehydrogenase (*IDH*) mutational status (**a**), cytogenetic risk (**b**), *JAK2* 46/1 haplotype status (**c**) or International Prognostic Scoring System risk category (**d**).

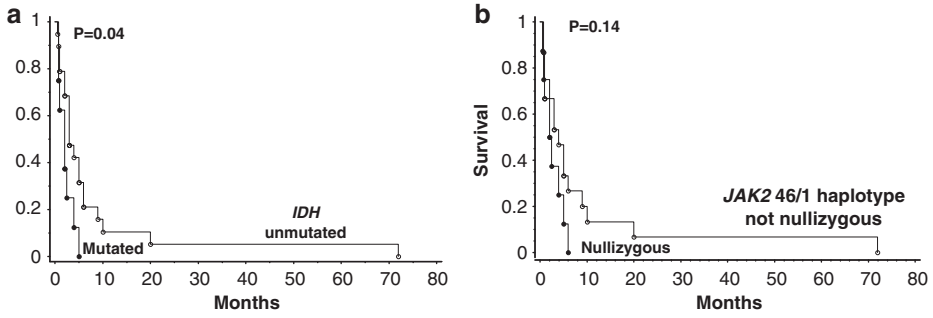

Figure 3 Survival curves of patients with blast-phase primary myelofibrosis stratified by their isocitrate dehydrogenase (*IDH*) mutational (**a**; $n=27$ including 8 mutated cases) or *JAK2* 46/1 haplotype (**b**; $n=23$ including 8 nullizygous cases) status.

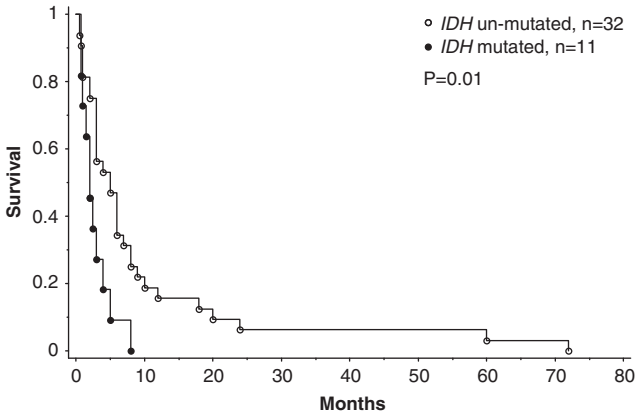

Figure 4 Survival curves of 43 patients with blast-phase myeloproliferative neoplasm stratified by their isocitrate dehydrogenase (*IDH*) mutational status.

cohort (Figure 4; $n=43$; $P=0.01$). In this instance, significance was sustained during multivariable analysis that included *JAK2* 46/1 haplotype as a covariate.

Discussion

IDH1 point mutations involving exon 4 occur in the majority (60–90%) of patients with low-grade gliomas and secondary glioblastomas, and always affect the amino acid arginine at position 132 (~93% R132H, 4% R132C, 2% R132S and <1% R132G, R132L or R132V).[8,22,23] These mutations are relatively infrequent in primary glioblastoma (~7%)[22] and are usually not seen in other solid tumors.[23,24] A small fraction (~4%) of glioma-associated *IDH* mutations involves *IDH2*, specifically the R132 analogous R172 residue on exon 4 (R172K, R172M, R172G, R172W).[23,25] *IDH* mutations in glioma are heterozygous, believed to constitute early genetic events and might be mutually exclusive of *EGFR* and *PTEN*, but not *TP53* mutations. Clinical correlates of *IDH* mutations in glioma include younger age, longer survival and reduced risk of disease progression after conventional therapy.[8,22,23,26,27]

The first study on *IDH* mutations in AML included 188 patients with primary AML and reported *IDH1*, but not *IDH2*, mutations in 8.5% ($n=16$) of the cases and 16% of those with normal karyotype: R132C in 8 patients, R132H in 7 and R132S in 1.[14] In a subsequent AML study of 493 patients,[28] 27 (5.5%) expressed *IDH1* mutations (37% R132C, 26% R132H, 19% R132S, 15% R132G and 4% R132L). In both studies,[14,28] *IDH1* mutations clustered with normal karyotype, *NPM1* mutations and trisomy 8. *IDH1* mutations are rare in pediatric AML.[29] More recently, *IDH2* mutations, affecting R172 (R172K)[12,13] or R140 (R140Q),[13] were also shown to occur in primary AML.[12,13] In one of these studies, *IDH1* or *IDH2* mutations were seen in 18 (23%) of 78 AML cases and the majority of the mutations (12 of 18) involved *IDH2*, primarily R140Q.[13] In general, survival in primary AML did not seem to be affected by the presence of *IDH* mutations.[13,14,28–30] However, more recent studies suggest that specific *IDH* mutation variants might be prognostically relevant in certain molecular subsets of AML.[31]

The first reports of *IDH* mutations in MPN came from three independent groups.[9,32,33] In one of these studies, *IDH1* mutations were seen in ~8% (5 of 63) of blast-phase MPN patients, mostly occurring in the absence of *TET2* and *ASXL1* mutations.[32] The second study was focused on blast-phase MPN that arose from *JAK2*-mutated chronic-phase MPN.[33] In this study, mutant *IDH* was seen in 5 (31%) of 16 blast-phase MPN (three cases with R132C and two with R140Q) and in none of the 180 PV or ET patients.[33] The third study from the Mayo Clinic included 200 MPN patients and showed *IDH* mutational frequencies of ~21% for blast-phase MPN, regardless of *JAK2* mutational status, and ~4% for PMF.[9] The specific *IDH1* mutations found in the particular study included R132C and R132S and the *IDH2* mutations R140Q and R140W.

The current study is an extension of the above-mentioned Mayo Clinic study and involves a large number of patients ($n=1473$) recruited from three major MPN centers of excellence. The results of the study clarify a number of issues regarding *IDH* mutations in MPN. First, the study provides robust incidence figures for *IDH1* and *IDH2* mutations across different disease stages of specific MPN variants. Accordingly, we now show that both *IDH1* and *IDH2* mutations can occur in chronic-phase ET, PV or PMF, although infrequently. Mutational frequency was equally low in post-PV/ET MF and this fact combined with the significantly higher mutation incidence observed in blast-phase disease suggests a pathogenetic contribution to leukemic but not fibrotic disease transformation. Two additional observations support this contention (i) complex karyotype was infrequently encountered in *IDH*-mutated blast-phase MPN, which suggests an independent pathogenetic contribution that might be tied to distinct molecular alterations, such as, for example, overexpression of the *APP* (*amyloid â (A4) precursor protein*) gene, which has previously been shown in AML to be associated with either complex karyotype or *IDH*R172 mutation[31] and (ii) the absence of mutual exclusivity between *IDH* and other MPN-associated mutations (for example, *TET2*, *MPL*), which is consistent with the suggestion that

the former are later-arising cooperating mutations that are more involved in disease progression rather than disease initiation.

The types of *IDH* mutations seen in our patients with MPN (mostly *IDH2*R140Q and *IDH1*R132S/C) are distinctly different than those seen in gliomas (mostly *IDH1*R132H) and more similar to those seen in AML, although *IDH1*R132H was significantly more prevalent in AML. Within the context of MPN, *IDH2*R140Q was over represented in chronic-phase ET and PV, whereas *IDH1* mutations were more prevalent in PMF and blast-phase MPN. More studies are needed to confirm this apparent trend. Regardless, there is currently no good explanation for the observed diversity in *IDH* mutation variants among gliomas and myeloid malignancies and current information suggests similar biological consequences.[13] Whether or not different *IDH* mutations carry different prognostic relevance in MPN is currently not known and we did not attempt to address the particular issue because of our relatively small number of informative cases. Of note, in a recent study of primary AML with normal karyotype, different types of *IDH* mutations appeared to variably influence disease-free survival and complete remission rates.[31]

One particularly interesting observation from the current study was the significant association between mutant *IDH* and *JAK2* non-46/1 haplotype. The latter phenomenon is further evidence for the *JAK2* mutation specificity of the previously described association between the *JAK2* 46/1 haplotype and MPN.[19,34,35] In other words, whereas *JAK2* exon 14[19,35] or exon 12[36] mutations have been shown to be associated with *JAK2* 46/1 haplotype, we did not see the same effect involving *MPL* mutations[34] (although others have shown otherwise),[37] and now show an association with *JAK2* non-46/1 haplotype for *IDH* mutations. This latter observation is also consistent with our previous report on the prognostically detrimental effect of *JAK2* non-46/1 haplotype in PMF;[19] it is possible that patients with PMF who are nullizygous for *JAK2* 46/1 haplotype are susceptible to additional adverse molecular events, such as *IDH* mutations, which might lead to biologically more aggressive disease. Consistent with this possible scenario, in the current study, the negative prognostic impact of mutant *IDH* was accounted for by the *JAK2* 46/1 genotype in PMF but not in blast-phase MPN, in which risk factors other than *JAK2* non-46/1 haplotype might have promoted the development of *IDH* mutations.

It is becoming increasingly evident that there are many more mutations than *JAK2* and *MPL* mutations in *BCR–ABL1*-negative MPN including those that involve *TET2*,[38,39] *ASXL1*,[40] *IDH1*,[32,33] *IDH2*,[9,33] *CBL*,[41] *IKZF1*[42] and *LNK*.[43] Some of these mutations might be later-arising and more prevalent in blast-phase disease. What is currently lacking is a composite evaluation (that is, concurrent analysis of all relevant mutations), which includes paired chronic- and blast-phase samples of a large number of patients with blast-phase MPN. Such an approach is essential for clarifying the individual pathogenetic or prognostic contribution of the aforementioned mutations and their chronological order of appearance. It is very likely that additional mutations in MPN will be described soon, but practical relevance in terms of either disease prognostication or value as drug targets has so far been limited.

Conflict of interest

The authors declare no conflict of interest.

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