Brief report

V617F mutation in *JAK2* is associated with poorer survival in idiopathic myelofibrosis

Peter J. Campbell, Martin Griesshammer, Konstanze Döhner, Hartmut Döhner, Rajko Kusec, Hans C. Hasselbalch, Thomas Stauffer Larsen, Niels Pallisgaard, Stéphane Giraudier, Marie-Caroline Le Bousse-Kerdilès, Christophe Desterke, Bernadette Guerton, Brigitte Dupriez, Dominique Bordessoule, Pierre Fenaux, Jean-Jacques Kiladjian, Jean-François Viallard, Jean Brière, Claire N. Harrison, Anthony R. Green, and John T. Reilly

Most patients with polycythemia vera and half with idiopathic myelofibrosis and essential thrombocythemia have an acquired V617F mutation in JAK2. Using sensitive polymerase chain reaction (PCR)–based methods, we genotyped 152 patients with idiopathic myelofibrosis to establish whether there were differences in presentation and outcome between those with and those without the mutation. Patients positive for V617F had higher neutrophil and white cell counts (*P* = .02) than did patients negative for V617F, but other diagnostic features were comparable between the 2 groups. Patients positive for V617F were less likely to require blood transfusion during follow-up (*P* = .03). Despite this, patients positive for V617F had poorer overall survival, even after correction for confounding factors (*P* = .01). (Blood. 2006;107:2098-2100)

Introduction

An acquired mutation in the *JAK2* gene has recently been described in a large proportion of patients with myeloproliferative disorders.[1-4] Most patients with polycythemia vera and about half those with idiopathic myelofibrosis (IMF) and essential thrombocythemia have the mutation. For patients with IMF, it is unknown whether there are differences between patients who are V617F-positive and -negative in their presenting features or survival. We therefore genotyped 152 patients with IMF for the V617F mutation and correlated their *JAK2* status with diagnostic features and outcome.

Study design

The study patients were treated in 14 centers in Europe and satisfied standard diagnostic criteria for idiopathic myelofibrosis.[5] We specifically excluded patients with secondary myelofibrosis, preceding polycythemia vera, or essential thrombocythemia and transitional myeloproliferative disorder. Sixteen patients were included in one of the original descriptions of the *JAK2* V617F mutation.[1] All centers had local ethics committee approval for the research, with overall approval from the Eastern Region Multicentre Research Ethics Committee, United Kingdom. All patients provided informed consent, in accordance with the Declaration of Helsinki. Information was collected on clinical and laboratory parameters at diagnosis, together with transfusion requirements and survival during follow-up. The Lille prognostic score was calculated as described.[6] Allele-specific polymerase chain reaction (PCR) or sequencing was used to screen genomic DNA (136 patients) or cDNA (16 patients) from peripheral blood or marrow, using previously published protocols.[1,7] Diagnostic variables were compared between patients who were V617F-positive and V617F-negative using the Wilcoxon rank-sum test for continuous variables and Pearson chi-squared test for categorical variables. Multivariate survival analysis was performed using Cox proportional hazards techniques.[8] The same Cox model resulted from both forward stepwise and backward elimination approaches.

Results and discussion

We screened 152 samples from patients with IMF for *JAK2* V617F using allele-specific PCR[1] or sequencing. In total, 83 patients were positive for the mutation, giving an overall frequency of the mutation of 54.6% (95% confidence interval, 50.7%-58.5%).

From the Department of Haematology, University of Cambridge, United Kingdom; the Department of Internal Medicine III, University of Ulm, Germany; the Department of Haematology and Clinical Chemistry, Merkur University Hospital, Zagreb, Croatia; the Department of Haematology and Pathology, Odense University Hospital, Denmark; the Institut National de la Santé et de la Recherche Médicale (INSERM) U362, Villejuif, and Laboratoire d'Hématologie, Henri Mondor Hospital, Créteil; INSERM U602, Villejuif, France; the Hématologie Clinique, Centre Hospitalier de Lens; the Hématologie Clinique, Center Hospitalier Régional Universitaire (CHRU) Limoges; the Hématologie Clinique, Hôpital Avicenne, Bobigny; the Hôpital du Haut-Lévêque, Pessac; the Department of Hematology, Hôpital Beaujon, Clichy, France; the Department of Haematology, St Thomas's Hospital, London, United Kingdom; and the Department of Haematology, Royal Hallamshire Hospital, Sheffield, United Kingdom.

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Reprints: John T. Reilly, Department of Haematology, Royal Hallamshire Hospital, Glossop Rd, Sheffield S10 2JF, United Kingdom; e-mail: j.t.reilly@sheffield.ac.uk.

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Table 1. Laboratory and clinical features at diagnosis of idiopathic myelofibrosis analyzed by presence or absence of the *JAK2* V617F mutation

	JAK2 V617F	*JAK2* wild-type	*P*
Total, no.	83	69	
Percentage (95% CI)	54.6 (50.7-58.5)	45.4 (41.5-49.3)	
Demographics			
Male, no. (%)	44 (53)	42 (61)	.3
Female, no. (%)	39 (47)	27 (39)	
Median age, y (10th-90th centile)	64 (48-77)	62 (43-77)	.3
Laboratory and clinical features at diagnosis			
LDH, U/L			.9
Mean ± SD	546 ± 374	501 ± 358	
Median (10th-90th centile)	440 (182-939)	451 (214-764)	
CD34, $\times 10^6$/L			.9
Mean ± SD	568 ± 724	620 ± 840	
Median (10th-90th centile)	292 (67-1332)	222 (22-1345)	
Blast cell count, $\times 10^9$/L			.5
Mean ± SD	0.25 ± 0.85	0.29 ± 0.71	
Median (10th-90th centile)	0.02 (0-0.56)	0.06 (0-0.67)	
Hemoglobin level, g/L			.1
Mean ± SD	110 ± 24	104 ± 22	
Median (10th-90th centile)	109 (73-139)	100 (81-132)	
White cell count, $\times 10^9$/L			.02
Mean ± SD	15.3 ± 15.4	10.1 ± 7.9	
Median (10th-90th centile)	10.0 (4.0-32.9)	7.2 (2.5-21.1)	
Neutrophil count, $\times 10^9$/L			.02
Mean ± SD	13.1 ± 15.7	8.1 ± 12.1	
Median (10th-90th centile)	7.7 (2.9-23.7)	4.7 (1.8-16.1)	
Platelet count, $\times 10^9$/L			.3
Mean ± SD	312 ± 252	254 ± 203	
Median (10th-90th centile)	227 (51-723)	232 (48-494)	
Spleen size, cm			.7
Mean ± SD	6.7 ± 5.2	7.4 ± 6.0	
Median (10th-90th centile)	7.0 (0-14.0)	5.0 (0-15.0)	
Cytogenetics, no.			.8
Normal	18	22	
Abnormal	16	15	
Lille score, no.			.06
0	36	17	
1	23	28	
2	6	5	
Transfusion required during follow-up, no.			.03
None	47	25	
Intermittent or regular	31	35	

Diagnostic clinical and laboratory features were compared between patients who were V617F-positive and -negative (Table 1). There were no significant differences in age, sex, hemoglobin levels, platelet count, peripheral blood blast counts, and spleen size. Similarly, there were no differences in lactate dehydrogenase (LDH) levels, CD34 counts, or frequency of abnormal karyotype, although data were only available on these variables for 48, 43, and 71 patients, respectively. In contrast, patients positive for V617F had significantly higher white cell counts than patients negative for V617F (median, 10.0 versus 7.2 $\times 10^9$/L; $P = .02$) and significantly higher neutrophil counts (median, 7.7 versus 4.7 $\times 10^9$/L; $P = .02$). This fact meant that the Lille prognostic scores tended to be lower (indicating better prognostic group) for patients positive for V617F ($P = .06$).

Patients positive for V617F were significantly less likely to require blood transfusions during follow-up than patients negative for V617F (Table 1). Of 78 patients positive for V617F in whom data were available, 47 (60%) did not require transfusions, compared with 25 (42%) of 60 patients negative for V617F ($P = .03$).

Patients positive for V617F had worse survival. With a median 38 months of follow-up, 17 patients positive for V617F and 12 patients negative for V617F died. To explore whether this apparent difference was due to the V617F mutation or explained by other potential confounding factors, we fitted multivariate Cox proportional hazards models for 110 patients from whom full clinical data were available (Table 2). When the effects of age and hemoglobin level were controlled for, patients positive for V617F had significantly poorer survival than patients negative for V617F (adjusted hazard ratio, 3.30; 95% confidence interval, 1.26-8.68; $P = .01$). Sex, Lille prognostic score, spleen

Table 2. Cox proportional hazards model of factors independently predictive of overall survival in myelofibrosis cohort

Variable	Adjusted hazard ratio	95% confidence interval	*P*
Hemoglobin level*	0.74	0.60-0.91	.004
Age†	1.61	1.10-2.36	.01
V617F	3.30	1.26-8.68	.01

Variables not independently associated with survival were sex, Lille prognostic score, white cell count, platelet count, and spleen size.

*Hazard ratio refers to proportional decrease in hazard ratio per 10 g/L (1 g/dL) increase in hemoglobin level at diagnosis.

†Hazard ratio refers to proportional increase in hazard ratio per 10-year increase in age.

size, platelet count, and white cell count were not independently predictive of survival. Some of the differences in survival may be explained by the fact that of the 6 patients with documented leukemic transformation, 5 were V617F-positive. None of the 6 patients had received busulphan, ^{32}P, or chlorambucil, and only one had received hydroxyurea before leukemic transformation, excluding therapy as an explanation for the differences in leukemia rates between patients who were V617F-positive or V617F-negative.

We found the *JAK2* V617F mutation present in just more than half of cases of idiopathic myelofibrosis, which accords well with previous estimates of its frequency in this disorder.[1-4] Patients positive for V617F had a significantly higher white cell count and neutrophil count than patients negative for V617F, suggesting that the mutation is associated with expansion of the myeloid lineage. In contrast, spleen size, platelet count, $CD34^+$ counts, and hemoglobin levels showed no significant differences between the V617F-positive and V617F-negative groups as a whole, but there was substantial interindividual variation in all of these parameters. This suggests that many of the clinical manifestations of IMF are caused by factors unrelated to the V617F mutation, and the high frequency of abnormal cytogenetics in both groups implies that other acquired abnormalities contribute substantially to the phenotype.

Interestingly, patients positive for V617F with IMF had less requirement for transfusion than patients negative for V617F. Given that the V617F mutation is common in polycythemia vera and that the mutation gives erythrocytosis in a murine model,[2] the V617F mutation may be able to partially protect patients with IMF against severe anemia.

In view of the lesser transfusion requirement for patients positive for V617F and the trend for better Lille prognostic score, it was interesting to find that patients positive for V617F had worse overall survival, particularly after correction for potential confounding factors. Not all the cases here were collected prospectively; therefore, survival analyses will be subject to length bias,[9] in which patients with more indolent disease are more likely to survive long enough to be registered in the cohort. However, unless there is a group of patients negative for V617F who do extremely poorly, length bias would, if anything, cause the differences in survival between patients who are V617F-positive and -negative to be underestimated. This emphasizes the need for a careful prospective study of the effects of the V617F mutation in IMF and suggests that the V617F mutation may be an important molecular prognostic marker for this disease.

Because most patients with IMF die of complications related to their disease, especially leukemic transformation and bone marrow failure,[6] the finding of poorer survival in patients positive for V617F suggests they have more aggressive disease. If confirmed in other studies, this would support the use of transplantation or experimental treatments in patients positive for V617F and underscore the role of *JAK2* as a potential therapeutic target in IMF.

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