JAK2 exon 12 mutations in polycythemia vera or idiopathic erythrocytosis

***JAK2* exon 12 mutations were detected in 4 out of 20 polycythemia vera and idiopathic erythrocytosis V617F-negative patients and were only present in the myeloid lineage. Initial hematologic data of these patients differ from those of V617F-positive patients, but there is no difference in thrombotic development and myelofibrotic transformation.**

Haematologica 2007; 92:1717-1718. DOI: 10.3324/haematol.12011

Recently, several gain-of-function mutations affecting exon 12 of the *JAK2* gene have been described in polycythemia vera (PV) V617F-negative patients and in some cases with idiopathic erythrocytosis (IE).[1-3] These patients display a clinical phenotype and bone marrow histologic features which differ from those observed in V617F-positive PV patients. This proposed distinctive myeloproliferative syndrome would be characterized by marked erythrocytosis, absence of leukocytosis or thrombocytosis, low serum erythropoietin levels and isolated erythroid hyperplasia in the bone marrow and, in some cases, endogenous erythroid colony growth. However, information about clinical outcome of these patients and hematopoietic cell lineage involvement of these mutations is limited.

We analyzed 29 patients diagnosed with PV or IE at three institutes, who were negative for the V617F *JAK2* mutation by allele specific PCR. The diagnosis of PV was centrally reviewed according to the criteria of the Polycythemia Vera Study Group.[4] Idiopathic erythrocytosis was diagnosed when patients having an absolute increase in red cell mass did not fulfil PV criteria and causes of secondary erythrocytosis were excluded after a careful workup, according to the guidelines for the diagnosis, investigation and management of polycythemia/erythrocytosis.[5] Nine out of 29 PV patients were positive for the V617F *JAK2* mutation by quantitative allele-specific PCR and were excluded from analysis. The remaining 20 patients (11 IE and 9 PV) were the subject of the study. Eighty-six V617F-positive PV patients, diagnosed and followed at the Hematology Department of the Hospital del Mar served as the PV control group for comparison. The study was approved by the local Ethics Committee of all participating centers and informed consent was obtained according to the Declaration of Helsinki.

JAK2 mutations in the exon 12 as assessed by direct sequencing were detected in 4 (1 PV, 3 IE) out of 20 (20%) patients negative for the V617F mutation. Three different exon 12 mutations were found: K539L which was detected in 2 patients, but was found to be from different nucleotide substitutions, N542_E543del and H538_K539delinsL (Figure 1). The first two mutations had been reported by Scott *et al.*, but the third has not yet been described.[1,2] All 3 mutations affecting exon 12 were found in heterozygosity. Confirmation of the K539L and N542_E543del was made by allele-specific PCR as previously described.[1] However, a negative result was obtained for the patient carrying the K539L mutation resulting from an AAA→CTA substitution. This emphasizes that mutations in exon 12 are irregularly allocated among several nucleotides and shows how difficult it is to detect these alterations by allele-specific PCR since primers specific for each mutation are needed.

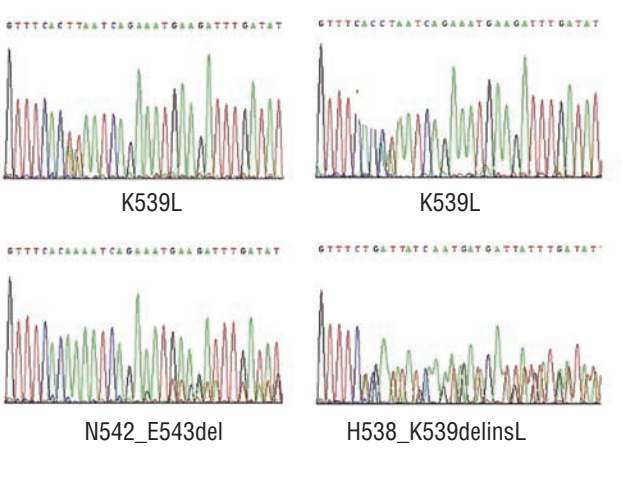

Figure 1. Sequencing chromatograms showing mutations affecting exon 12 of the JAK2 gene. The upper panels show a lysine to leucine substitution at amino acid position 539 (K539L) resulting from a AAA→TTA exchange (left) or from a AAA→CTA exchange (right). Lower panels show the deletion of amino acids 542 and 543 (N542_E543del) (left) and the deletion of amino acids 538 and 539 with the insertion of a leucine (H538_K539delinsL) (right).

To determine which blood cell populations were affected by exon 12 mutations, granulocytes, T lymphocytes, B lymphocytes, natural killer (NK) cells and monocytes were purified by cell sorting on a FACSVantage flow cytometer and cell sorter (Becton Dickinson, San José, CA, USA) with a cell purity >95% for all fractions. Platelet-rich plasma was obtained by centrifugation. In all 4 patients, mutations affecting exon 12 were detected in granulocytes and platelets. These mutations were not detected in B and T lymphocytes or NK cells, but were detected in monocytes in 2 out of 4 cases. To our knowledge, this is the first report that demonstrates a predominant myeloid lineage involvement of *JAK2* exon 12 mutations.

In 7 out of 16 patients negative for both V617F and exon 12 mutations, the whole codifying sequence of the *JAK2* gene was analyzed by direct sequencing but no pathogenic mutations were detected. The lack of mutations in the *JAK2* gene in this subset of patients suggests the possible existence of other genetic alterations.

The main clinicohematologic data at diagnosis of the 4 patients with exon 12 mutations are shown in Table 1. The 4 patients with exon 12 mutations were of female gender in contrast to 38 out of 86 (44%) V617F-positive PV patients (p=0.04). Patients with exon 12 mutations showed significantly lower platelet counts at diagnosis (231±53 *vs.* 601±286×10^9/L, p=0.001) and lower leukocyte cell counts (6.9±2.3 *vs.* 11.5±4.6×10^9/L, p=0.02) than V617F-positive PV patients, in agreement with previous reports.[1,2] No significant differences were observed in age and hemoglobin level. PRV1 was overexpressed in all 4 patients. Bone marrow biopsy at diagnosis in 3 patients showed an increased cellularity with erythroid and megakaryocytic hyperplasia.

The remaining 16 patients (9 PV, 7 IE), negative for both V617F and exon 12 JAK2 mutations, were 3 women

Table 1. Main clinicohematologic data at diagnosis of 4 patients with JAK2 exon 12 mutations.

	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*
Initial diagnosis	PV	IE	IE	IE
Age/sex	76/F	73/F	40/F	52/F
JAK2 mutation	K539L	K539L	H538_K539delinsL	N542_E543del
Hemoglobin, g/L	151	176	218	197
WBC, $\times10^9$/L	4.6	4.2	8.6	9.1
Platelets, $\times10^9$/L	217	301	173	235
Epo level, IU/L	2.2	1.1	47	2
Splenomegaly	Absent	Absent	Absent	Absent
Karyotype	47 XX, +9	46 XX	46 XX	46 XX
EEC	Yes	ND	ND	ND
PRV-1 overexpression	Yes	Yes	Yes	Yes
Thrombosis	No	Yes*	Yes**	No
Transformation to myelofibrosis	No	No	Yes	No
Major bleeding	No	No	No	No

*PV: polycythemia vera; IE: idiopathic erythrocytosis; F: female; EEC: erythropoietin-independent erythroid colonies growth; ND: not determined; *deep venous thrombosis; **mesenteric arterial thrombosis.*

and 13 men. There were no significant differences in age, hemoglobin level, leukocyte counts, platelet counts and serum erythropoietin levels when compared with patients carrying exon 12 mutations. When compared with the V617F-positive PV patients, no significant differences were observed concerning age, sex and leukocyte counts. However, patients negative for both V617F and exon 12 JAK2 mutations showed significantly lower platelet counts at diagnosis (308±243 *vs.* 601±286×10^9/L, p=0.001) and higher hemoglobin level (187±16 *vs.* 174±25 g/L, p=0.05) than V617F-positive PV patients.

After a median follow-up of six years (range 5-23 years), of the 4 patients with exon 12 mutations, 2 developed major thrombotic events at 10 months and 19 years after diagnosis (Table 1). The remaining 2 patients did not present thrombotic events after a follow-up of 4.3 and 5.6 years respectively. Transformation to myelofibrosis was observed in 1 case at 20 years after PV diagnosis (patient 3). All 4 patients remain alive at last follow-up. Given these results, it seems that the clinical outcome of patients with exon 12 mutations would be similar to that reported for classical PV patients.[6]

In conclusion, mutations in exon 12 are detected in a variable proportion of *JAK2* V617F-negative PV or IE patients and mainly affect the myeloid lineages. Although the initial hematologic data differ from V617F-positive patients, thrombotic development and myelofibrotic transformation during follow-up are similar to classical *JAK2* V617F-positive PV patients.

Luz Martínez-Avilés,^ Carles Besses,° Alberto Álvarez-Larrán,° Francisco Cervantes,# Juan Carlos Hernández-Boluda,@ Beatriz Bellosillo*§*

LM-A and CB equally contributed to this study

Departments of Pathology and Clinical Hematology° Hospital del Mar, IMAS, Barcelona; Department of Hematology, Hospital Clínic, University of Barcelona;# Department of Hematology, Hospital Clínic, Valencia;@ Universitat Autònoma de Barcelona,^ Universitat Pompeu Fabra, Spain§*

Acknowledgments: we thank Oscar Fornas for his contribution to cell sorting experiments and Mercè Musset and Erica Torres for excellent technical assistance.

Key words: JAK2 mutation, exon 12, V617F mutation, PV, erythrocytosis.

Correspondence: Carles Besses, MD, PhD, Department of Clinical Hematology, Hospital del Mar, Passeig Marítim 25-29 08003, Barcelona, Spain. Phone: international +34.9.32483341. Fax: international +34.9.32483131. E-mail: 92717@imas.imim.es

References

1. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med 2007;356:459-68.
2. Pardanani A, Lasho TL, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. Leukemia 2007;21:1960-3.
3. Percy MJ, Scott LM, Erber WN, Harrison CN, Reilly JT, Jones FGC, et al. JAK2 exon 12 mutations occur frequently in idiopathic erythrocytosis patients with low serum erythropoietin levels. Haematologica 2007; 92[Suppl 1]: 333-4[Abstract].
4. Pearson TC. Evaluation of diagnostic criteria in polycythemia vera. Semin Hematol 2001;38:21-4.
5. McMullin MF, Bareford D, Campbell P, Green AR, Harrison C, Hunt B, et al. Guidelines for the diagnosis, investigation and management of polycythaemia/erythrocytosis. Br J Haematol 2005;130:174-95.
6. Marchioli R, Finazzi G, Landolfi R, Kutti J, Gisslinger H, Patrono C, et al. Vascular and neoplastic risk in a large