Brief report

Somatic mutations of *JAK2* exon 12 in patients with *JAK2* (V617F)-negative myeloproliferative disorders

Daniela Pietra,[1] Sai Li,[2] Angela Brisci,[3] Francesco Passamonti,[1] Elisa Rumi,[1] Alexandre Theocharides,[2] Maurizio Ferrari,[3-5] Heinz Gisslinger,[6] Robert Kralovics,[6,7] Laura Cremonesi,[3,4] Radek Skoda,[2] and Mario Cazzola[1]

[1]Department of Hematology, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo and University of Pavia, Pavia, Italy; [2]Department of Research, Experimental Hematology, Basel University Hospital, Basel, Switzerland; [3]Genomic Unit for the Diagnosis of Human Pathologies, San Raffaele Scientific Institute, Milan, Italy; [4]Diagnostica e Ricerca San Raffaele, Milan, Italy; [5]Università Vita-Salute San Raffaele, Milan, Italy; [6]Department of Internal Medicine I, Division of Hematology and Blood Coagulation, Medical University of Vienna, Vienna, Austria; and [7]Center for Molecular Medicine (CeMM), Austrian Academy of Sciences, Vienna, Austria

We searched for *JAK2* exon 12 mutations in patients with *JAK2* (V617F)-negative myeloproliferative disorders. Seventeen patients with polycythemia vera (PV), including 15 sporadic cases and 2 familial cases, carried deletions or duplications of exon 12 in circulating granulocytes but not in T lymphocytes. Two of the 8 mutations detected were novel, and the most frequent ones were N542-E543del and E543-D544del. Most patients with PV carrying an exon 12 mutation had isolated erythrocytosis at clinical onset, unlike patients with *JAK2* (V617F)-positive PV, most of whom had also elevations in white blood cell and/or platelet counts. Both patients with familial PV carrying an exon 12 mutation had an affected sibling with *JAK2* (V617F)-positive PV. Thus, several somatic mutations of *JAK2* exon 12 can be found in a myeloproliferative disorder that is mainly characterized by erythrocytosis. Moreover, a genetic predisposition to acquisition of different *JAK2* mutations is inherited in families with myeloproliferative disorders. (Blood. 2008;111:1686-1689)

Introduction

The identification of a unique gain-of-function mutation of the *Janus kinase 2* (*JAK2*) gene in patients with chronic myeloproliferative disorders[1-5] has provided clinicians with a very useful diagnostic tool. In fact, the *JAK2* (V617F) mutation can be detected in approximately 95% of patients with polycythemia vera (PV), and in more than half of those with essential thrombocythemia (ET) or primary myelofibrosis (PMF).[1,6-8] While only a minority of sporadic patients with PV do not carry *JAK2* (V617F), the proportion of familial cases of PV that are negative is definitely higher.[9,10] Moreover, most patients with idiopathic erythrocytosis do not carry *JAK2* (V617F) in circulating granulocytes.[11]

The *JAK2* (V617F) mutation involves the exon 14, which encodes a part of the JH2 auto-inhibitory domain of the JAK2 kinase. Scott and coworkers[12] recently described mutations of *JAK2* exon 12 in *JAK2* (V617F)-negative patients with PV or idiopathic erythrocytosis. Their findings have been confirmed by other studies.[13-17] Using cellular models, it has been shown that exon 12 mutations can activate pathways associated with erythropoietin signaling, and in a murine model one of these mutations resulted in a myeloproliferative phenotype.[12]

In this work, we searched for exon 12 mutations in patients with *JAK2* (V617F)-negative myeloproliferative disorders.

Methods

We studied 147 patients with *JAK2* (V617F)-negative myeloproliferative disorders. These investigations were approved by the local ethics committees of Pavia, Basel, and Vienna: the procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000, and samples were obtained from subjects after written informed consent was obtained in accordance with the Declaration of Helsinki. The 2001 World Health Organization (WHO) criteria[18] were applied for the diagnosis of PV and ET, while the Italian consensus criteria were used for the diagnosis of PMF.[19] Of the 147 patients reported in this work, 26 had sporadic PV, 11 had familial PV,[9,10] 75 had sporadic ET, and 35 had sporadic PMF.

The isolation of granulocytes and T lymphocytes was performed as previously described.[6,20] Quantitative real-time polymerase chain reaction (qRT-PCR)–based allelic discrimination assays[6,7] were used for the detection of the *JAK2* (V617F) mutation.

For exon 12 mutation screening, primers were designed to amplify a region of 453 base pairs (bp) containing the 128 bp of the exon 12 as described in Document S1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article). Direct sequencing was performed using the Big Dye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and an ABI Prism 3130 Genetic Analyzer (Applied Biosystems).

Submitted July 16, 2007; accepted October 28, 2007. Prepublished online as *Blood* First Edition paper, November 6, 2007; DOI 10.1182/blood-2007-07-101576.

D.P. and S.L. contributed equally to this work.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Table 1. Clinical and hematologic characteristics at diagnosis for the 17 patients carrying a *JAK2* exon 12 mutation.

UPN	Sex/ age	Clinical diagnosis	Hemoglobin level, g/L	WBC count, 10^9/L	PLT count, 10^9/L	Serum Epo, mU/mL	Palpable splenomegaly	*JAK2* exon 12 granulocytes		
								DNA alteration	Predicted amino acid change	Pattern on sequencing*
PaS39A3V	M/80	PV	185	11.8	514	<2	Yes	1627-1632del6	E543-D544del	Het
PaS33A4V	M/40	PV	238	6.8	308	<2	No	1606-1638dup33	V536-I546dup11	Het
PaS90A6V	F/47	PV	191	7.3	261	<2	No	1624-1629del6	N542-E543del	Het
PaS25A4V	F/35	PV	170	10.0	274	<2	No	1608-1640dup33	F537-I546dup10+F547L	Hom
PaS116A6V	M/53	PV	220	5.8	366	3.2	No	1624-1629del6	N542-E543del	Het
PaS1A7V	F/76	PV	172	6.1	788	3.5	No	1622-1627del6	R541-E543delinsK	Het
PaS23A5V	F/78	PV	175	4.6	339	<2	No	1627-1632del6	E543-D544del	Het
PaS6A95V	M/47	PV	207	6.0	360	3.0	No	1624-1629del6	N542-E543del	Het
PaS1A5V	F/53	Familial PV	167	4.2	306	3.6	No	1622-1627del6	R541-E543delinsK	Het
PaS12A0V	M/58	Familial PV	230	5.7	144	<2	No	1624-1629del6	N542-E543del	Het
Ba002	F/46	PV	226	6.0	215	<2	Yes	1620-1621del2,1626-1629del4	I540-E543delinsMK	Het
Ba041	M/43	PV	214	7.5	297	17.2	No	1611-1616del6	F537-K539delinsL	Het
Ba138	F/69	PV	194	8.6	102	<2	No	1613-1615del3, A1616T	H538-K539delinsL	Het
Ba166	F/46	PV	170	9.0	956	<2	No	1627-1632del6	E543-D544del	Het
Ba221	F/36	PV	171	9.3	689	3.0	Yes	1627-1632del6	E543-D544del	Het
Vi0064	F/64	PV	199	11.2	700	<2.5	No	1627-1632del6	E543-D544del	Het
Vi0327	F/70	PV	177	5.7	253	ND	No	1627-1632del6	E543-D544del	Het

All T-cell DNA was wild-type. Reference ranges are as follows Hb, 130 to 170 g/L (males), 120 to 160 g/L (females); WBC count, 4 to 11 $\times$ 10^9/L; PLT, 100 to 400 $\times$ 10^9/L; serum Epo, 5 to 30 mU/mL.
UPN indicates unique patient number (Pa, Pavia; Ba, Basel; Vi, Vienna); PV, polycythemia vera; Epo, erythropoietin; ND, not determined; Het, heterozygous; and Hom, homozygous.
* Analysis of heights of wild-type and mutant peaks showed proportions of mutant alleles ranging from approximately 10% to approximately 90% of total alleles.

Results and discussion

Of the 147 patients with *JAK2* (V617F)-negative myeloproliferative disorders, 17 carried mutations of exon 12 in circulating granulocytes. Specifically, exon 12 mutations were detected in 15/26 sporadic cases and in 2/11 familial cases of PV. All positive patients met the revised WHO criteria[21] for PV. Demographic and hematologic characteristics of these patients, and details of their mutations are reported in Table 1. A comparison between patients with sporadic PV who carried a *JAK2* exon 12 mutation and those who did not is reported in Table S1.

Twelve of the 17 patients with PV carrying a *JAK2* exon 12 mutation had isolated erythrocytosis at presentation, with WBC counts less than or equal to 12 $\times$ 10^9/L, and with platelet counts less than or equal to 400 $\times$ 10^9/L. This frequency (12/17 or 71%) was significantly higher than that observed in a series of 92 patients diagnosed with *JAK2* (V617F)-positive PV at the Department of Hematology, Pavia, Italy (20/92 or 22%, $P < .001$). All patients but one carrying a *JAK2* exon 12 mutation had low serum erythropoietin levels, indicating a combination of absolute erythrocytosis and suppressed endogenous erythropoietin production.[22]

Overall, 8 different mutations were found in this study, and the 2 duplications are novel at the time of this writing. All mutations were detected in circulating granulocytes but not in T lymphocytes, indicating that these were acquired somatic mutations. The pattern on sequencing was heterozygous in 16 of 17 patients (Table 1): representative patterns are reported in Figure S1.

Mutations spanned from base 1606 to 1640, and the 2 duplications modified the rest of the sequence by adding 33 bp (Figure 1, Figure S2). In terms of protein, deletions predicted amino acid changes spanning from phenylalanine 537 to aspartic acid 544, while duplications predicted changes from phenylalanine 547 onward within the JH2 pseudokinase domain. Three categories of molecular lesions were identified (Figure 1): (i) those involving a K539L substitution; (ii) those involving a deletion of glutamic acid 543 (E543del); and (iii) amino acid duplications involving a substitution of phenylalanine 547. Based on data of this study and findings of previous reports,[12-17] the most frequent exon 12 mutations detected so far are N542-E543del, F537-K539delinsL, and E543-D544del.

Two of 11 patients with *JAK2* (V617F)-negative familial PV carried a mutation of exon 12 (Figure 2). Both patients had an affected sibling with *JAK2* (V617F)-positive PV. While subjects carrying an exon 12 mutation showed isolated erythrocytosis, their *JAK2* (V617F)-positive siblings had also thrombocytosis.

The present study confirms previous observations on *JAK2* exon 12 mutations in myeloproliferative disorders, describes 2 novel mutations, and provides evidence for intrafamily discordance of *JAK2* mutation type in familial disorders.

The seminal study by Scott and coworkers[12] led to the conclusion that *JAK2* exon 12 mutations define a distinctive myeloproliferative syndrome that affects patients who currently receive a diagnosis of PV or idiopathic erythrocytosis. Our findings confirm that these patients indeed have a myeloproliferative syndrome presenting mainly with isolated erythrocytosis associated with suppressed erythropoietin production. Exon 12 mutations were shown to be associated with peculiar biochemical abnormalities of JAK2 signaling,[12] which likely favor erythrocytosis.

Of our 17 patients carrying exon 12 mutations, all but one had heterozygous patterns on sequencing (Table 1 and Figure S1). In

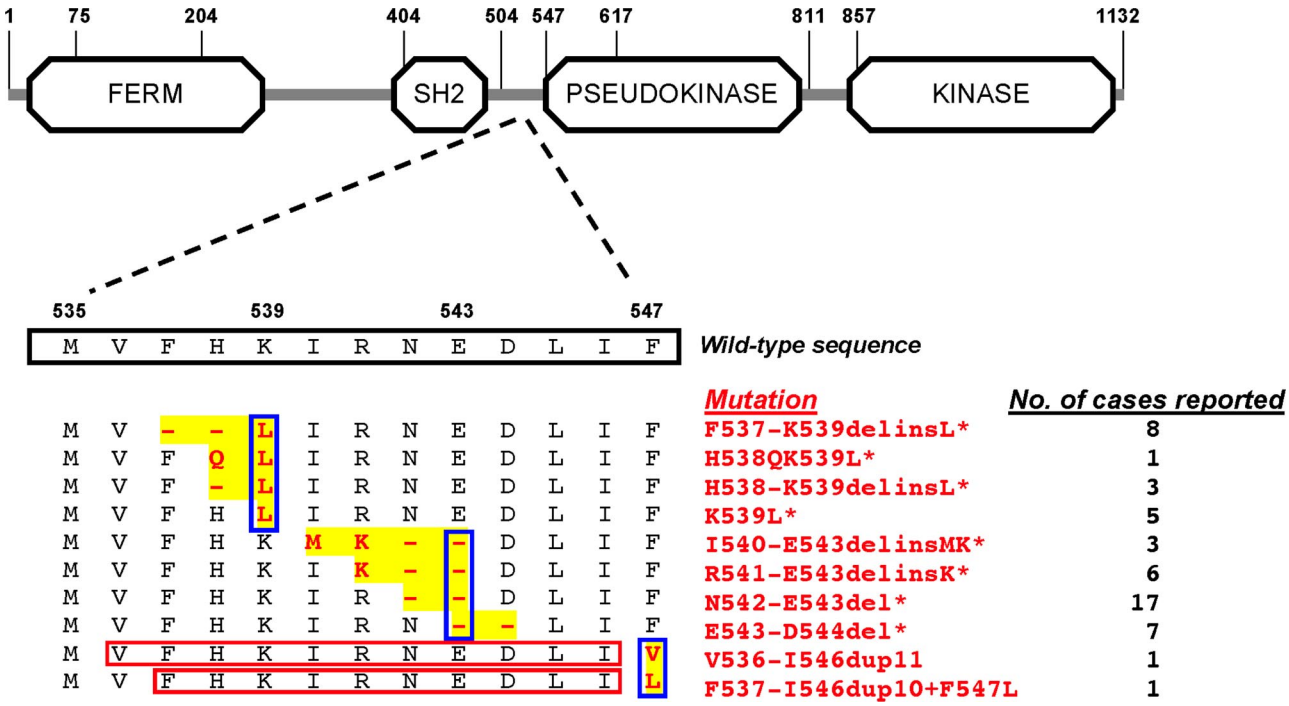

Figure 1. Schematic representation of the *JAK2* gene with the wild-type sequence of amino acids encoded by exon 12, and the exon 12 mutations detected so far. Regions of rearrangement are highlighted in yellow, while mutations are shown in red; the red rectangles indicate the duplicated sequences. All mutations so far identified in patients with PV or idiopathic erythrocytosis are illustrated: those previously reported[12-17] are marked with an asterisk. Blue rectangles indicate the 3 types of mutations: (i) those involving a K539L substitution; (ii) those involving a deletion of glutamic acid 543 (E543del); (iii) and duplications involving a substitution of phenylalanine 547. On the right, for each mutation the number of cases reported so far is indicated. Information on JAK2 domains is from URL: http://smart.embl-heidelberg.de.

our original report on the unique *JAK2* (V617F) mutation,[4] 48 of 83 (58%) of *JAK2* (V617F)-positive patients with PV showed a heterozygous pattern on sequencing, while the remaining 35 of 83 (42%) had a homozygous pattern. These observations may suggest that mitotic recombination (ie, the mechanism leading to homozygosity,[4]) is more likely to occur in PV patients carrying *JAK2* (V617F) than in those with exon 12 mutations.

Variable proportions of patients with *JAK2* (V617F)-negative PV that carry an exon 12 mutation have been reported.[12,13,15-17] We used direct sequencing, which allowed us to identify novel mutations but is unlikely to detect percentages of mutant alleles lower than 10%. At least a portion of our negative PV patients (Table S1) might therefore carry low percentages of mutant alleles in granulocytes. The inability of direct sequencing to detect them may justify the discrepancy between our findings and those of previous reports.[12,13]

Studies of families with myeloproliferative disorders[9,10,23,24] showed that *JAK2* (V617F) represents an acquired somatic mutation in familial cases as it does in sporadic cases,[25] and that a genetic predisposition to acquisition of *JAK2* (V617F) is inherited. The current study indicates that this predisposition regards any type of *JAK2* mutations, not just *JAK2* (V617F). It might be hypothesized that these individuals have defective mechanisms for repairing or neutralizing spontaneous mutations, so that those involving gain-of-function emerge and lead to clonal proliferation. In addition, if one considers the 2 families reported in Figure 2, only few individuals in these families have a disease phenotype. This makes the distinction between familial and sporadic cases more subtle, and raises the question whether a genetic predisposition is present also in the so-called sporadic cases.

Acknowledgments

We thank Tiina Berg, Sabrina Boggi, and Roland Jäger for their technical assistance.

This study was supported by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC), Fondazione Cariplo, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Ministry of University and Research, and Alleanza Contro il Cancro, Italy (M.C.); grants from the Swiss National Science Foundation (310 000-108 006/1), the Swiss Cancer League (OCS-01742-08-2005), and the Krebsliga beider Basel (R.S.); and grants from the Initiative for Cancer Research of the Medical University of Vienna and the Privatstiftung Unruhe (R.K.).

Authorship

Contribution: M.C. and R.S. designed research, analyzed data, and wrote the paper; F.P., E.R., A.T., and H.G. collected and analyzed clinical data; and D.P., S.L, A.B., M.F., R.K., and L.C. performed molecular investigations.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Prof Mario Cazzola, Department of Hematology, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy; e-mail: mario.cazzola@unipv.it.

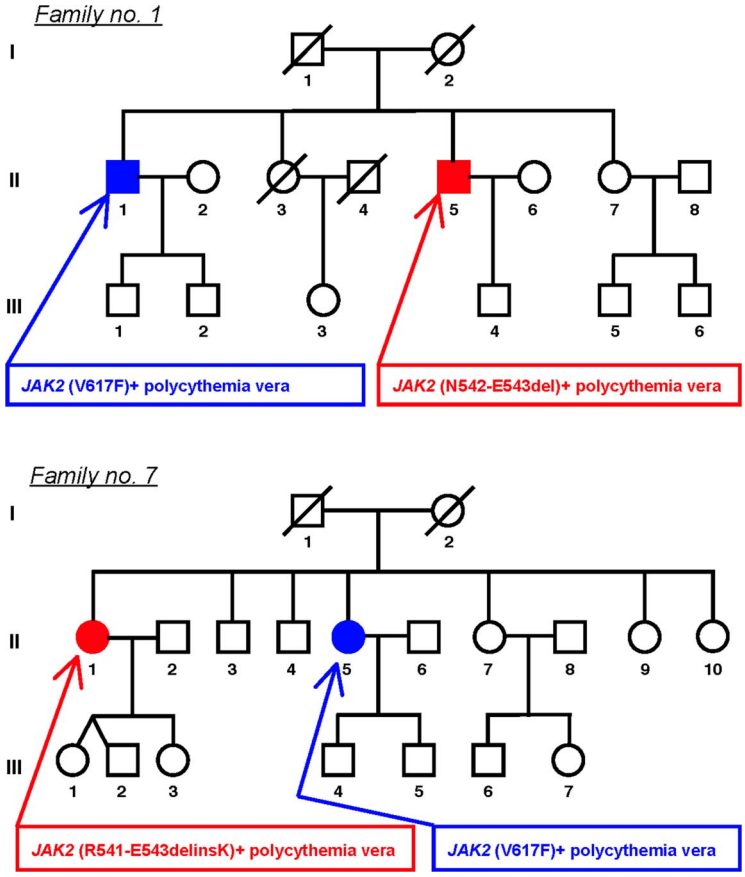

Figure 2. Pedigrees of 2 families with myeloproliferative disorders characterized by intra-family discordance of *JAK2* mutation type. Each family member is identified by a pedigree number. Circles represent female family members; squares male family members; filled symbols members with phenotypically expressed myeloproliferative disorder; slashes members who had died. In family 1, 2 brothers (II-1 and II-5) had PV. Subject II-1 presented at the age of 57 with ischemic stroke, and at the age of 63 he was diagnosed with PV (erythrocytosis and thrombocytosis). This patient was later found to be *JAK2* (V617F)-positive, and his granulocyte mutant alleles increased from 6% to 20% over time. He died at the age 69 of pulmonary embolism despite low-dose aspirin and cytoreductive treatment. His brother (subject II-5) was found to have erythrocytosis on routine blood counts at the age of 58; a diagnosis of PV was made, and venesection therapy was started. He was found to carry the N542-E543del mutation in circulating granulocytes. In family 7, 2 sisters (II-1 and II-5) had PV. Subject II-5 presented at the age of 59 with erythrocytosis and thrombocytosis. When she was admitted to the Department of Hematology, Pavia, Italy, at the age of 78, she was found to have post-PV myelofibrosis: she had splenomegaly (below the umbilical line), elevated circulating $CD34^+$ cell count (244×10^6/L), and 91% granulocyte *JAK2* (V617F) mutant alleles. Her sister (subject II-1) presented at the age of 53 with erythrocytosis, was diagnosed with PV, and started venesection therapy. She was found to carry the R541-E543delinsK mutation in circulating granulocytes.

References

1. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365:1054-1061.
2. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;7:387-397.
3. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005;434:1144-1148.
4. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352:1779-1790.
5. Jones AV, Kreil S, Zoi K, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood. 2005;106:2162-2168.
6. Passamonti F, Rumi E, Pietra D, et al. Relation between JAK2 (V617F) mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders. Blood. 2006;107:3676-3682.
7. Lippert E, Boissinot M, Kralovics R, et al. The JAK2-V617F mutation is frequently present at diagnosis in patients with essential thrombocythemia and polycythemia vera. Blood. 2006;108:1865-1867.
8. Tefferi A, Lasho TL, Schwager SM, et al. The clinical phenotype of wild-type, heterozygous, and homozygous JAK2V617F in polycythemia vera. Cancer. 2006;106:631-635.
9. Rumi E, Passamonti F, Pietra D, et al. JAK2 (V617F) as an acquired somatic mutation and a secondary genetic event associated with disease progression in familial myeloproliferative disorders. Cancer. 2006;107:2206-2211.
10. Rumi E, Passamonti F, Della Porta MG, et al. Familial chronic myeloproliferative disorders: clinical phenotype and evidence of disease anticipation. J Clin Oncol. 2007;25:5630-5635.
11. Percy MJ, Jones FG, Green AR, Reilly JT, McMullin MF. The incidence of the JAK2 V617F mutation in patients with idiopathic erythrocytosis. Haematologica. 2006;91:413-414.
12. Scott LM, Tong W, Levine RL, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med. 2007;356:459-468.
13. 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;21:1960-1963.
14. Percy MJ, Scott LM, Erber WN, et al. The frequency of JAK2 exon 12 mutations in idiopathic erythrocytosis patients with low serum erythropoietin levels. Haematologica. 2007;92:1607-1614.
15. Martinez-Aviles L, Besses C, Alvarez-Larran A, Cervantes F, Hernandez-Boluda JC, Bellosillo B. JAK2 exon 12 mutations in patients with polycythemia vera or idiopathic erythrocytosis. Haematologica. 2007;92:1717-1718.
16. Butcher CM, Hahn U, To LB, et al. Two novel JAK2 exon 12 mutations in JAK2V617F-negative polycythaemia vera patients. Leukemia. Prepublished on October 4, 2007 as DOI 10.1038/sj.leu.2404971.
17. Williams DM, Kim AH, Rogers O, Spivak JL, Moliterno AR. Phenotypic variations and new mutations in JAK2 V617F-negative polycythemia vera, erythrocytosis, and idiopathic myelofibrosis. Exp Hematol. 2007;35:1641-1646.
18. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002;100:2292-2302.
19. Barosi G, Ambrosetti A, Finelli C, et al. The Italian Consensus Conference on Diagnostic Criteria for Myelofibrosis with Myeloid Metaplasia. Br J Haematol. 1999;104:730-737.
20. Kralovics R, Teo SS, Buser AS, et al. Altered gene expression in myeloproliferative disorders correlates with activation of signaling by the V617F mutation of Jak2. Blood. 2005;106:3374-3376.
21. Tefferi A, Thiele J, Orazi A, et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. Blood. 2007;110:1092-1097.
22. Girodon F, Lippert E, Mossuz P, et al. JAK2V617F detection and dosage of serum erythropoietin: first steps of the diagnostic work-up for patients consulting for elevated hematocrit. Haematologica. 2007;92:431-432.
23. Bellanné-Chantelot C, Chaumarel I, Labopin M, et al. Genetic and clinical implications of the Val617Phe JAK2 mutation in 72 families with myeloproliferative disorders. Blood. 2006;108:346-352.
24. Pardanani A, Lasho T, McClure R, Lacy M, Tefferi A. Discordant distribution of JAK2V617F mutation in siblings with familial myeloproliferative disorders. Blood. 2006;107:4572-4573.
25. Cazzola M, Skoda R. Gain of function, loss of control - a molecular basis for chronic myeloproliferative disorders. Haematologica. 2005;90:871-874.