Int J Hematol
DOI 10.1007/s12185-012-1169-8

ORIGINAL ARTICLE

The *JAK2* 46/1 haplotype is a risk factor for myeloproliferative neoplasms in Chinese patients

Xinju Zhang · Tingting Hu · Zhiyuan Wu · Zhihua Kang · Weiwei Liu · Ming Guan

Received: 4 June 2012 / Revised: 23 August 2012 / Accepted: 27 August 2012

Abstract The presence of *JAK2* V617F is associated with an inherited *JAK2* 46/1 haplotype, a risk factor for myeloproliferative neoplasms (MPN) in Caucasian populations. Whether the *JAK2* 46/1 haplotype is also a risk factor in the Chinese population is unknown. We assessed for the *JAK2* 46/1 haplotype and *JAK2* V617F mutation in 225 MPN patients and 226 controls using a tagged SNP rs12340895. The allele frequencies of the *JAK2* 46/1 haplotype were distinct among different subtypes of MPN patients. The allele frequency was significantly higher in polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) than that in controls, with PV patients having the highest allele frequency (0.58, $P = 6.00\text{E}{-15}$). The distribution of rs12340895 genotypes in the *JAK2* V617F mutated MPN patients was significantly different from that in controls ($P = 1.67\text{E}{-15}$). The percentage of GG genotype in controls was 2.2 %, but 31.0 % in *JAK2* V617F-positive MPN patients. All the PV, ET, and PMF patients with the GG genotype also exhibited the V617F mutation. Compared to that of controls, the difference in genotype distribution in PV patients was the most significant ($P = 4.83\text{E}{-21}$), followed by ET ($P = 2.07\text{E}{-05}$) and PMF ($P = 1.99\text{E}{-04}$). Our results suggest that the *JAK2* 46/1 haplotype is a risk factor for MPN in the Chinese population, and patients with GG genotype in rs12340895 locus are susceptible to *JAK2* V617F mutation.

Keywords *JAK2* V617F · SNP · Myeloproliferative neoplasms

Introduction

Myeloproliferative neoplasm (MPN) arises from the precursors of the myeloid lineage and represents a group of diseases of the bone marrow, in which excessive cells are produced. Somatic acquisition of genetic aberrations may be one of the pathogenic mechanisms, and it is widely believed that inherited genetic factors play an important role in the development of MPNs [1, 2]; however, the underlying molecular mechanisms remain to be determined.

Identification of the V617F mutation of the *JAK2* gene (*JAK2* V617F) is a breakthrough in the understanding of the molecular mechanisms of the majority of MPN diseases [3–6]. *JAK2* is a non-receptor tyrosine kinase involved in a specific subset of cytokine receptor signaling pathways. In the JAK-STAT signaling pathway, *JAK2* is the key component for the differentiation and survival of the myeloid lineage [3–6]. The *JAK2* V617F mutation renders bone marrow cells hypersensitive to cytokine stimulation and causes abnormal growth of hematopoietic stem cells, consequently leading to myeloproliferative tumors. It has been designated as a molecular marker for the diagnosis of polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) according to the latest World Health Organization Classification of MPNs [7]. However, this somatic mutation (*JAK2* V617F) cannot explain the molecular mechanisms for the 5- to 7-fold

X. Zhang and T. Hu equally contributed to this study.

X. Zhang · M. Guan (✉)
Department of Central Laboratory, Huashan Hospital, Shanghai Medical College, Fudan University, 12 Central Urumqi Road, Shanghai 200040, People's Republic of China
e-mail: guanming88@yahoo.com

T. Hu · Z. Wu · Z. Kang · W. Liu (✉) · M. Guan
Department of Laboratory Medicine, Huashan Hospital, Shanghai Medical College, Fudan University, 12 Central Urumqi Road, Shanghai 200040, People's Republic of China
e-mail: huashanvivian@yahoo.com.cn

Published online: 29 September 2012

increase of MPNs in the first-degree relatives of MPN patients [8].

Recent studies clearly demonstrated that the presence of *JAK2* V617F is associated with an inherited *JAK2* haplotype designated as "46/1" haplotype, and the *JAK2* 46/1 haplotype is a risk factor for myeloproliferative neoplasms in Caucasian populations [3, 9, 10]. However, whether the *JAK2* 46/1 haplotype is a risk factor in Chinese individuals is unclear. To investigate the role of *JAK2* 46/1 haplotype in Chinese MPN patients, we conducted a case/control study in Chinese. Our data demonstrated that the *JAK2* 46/1 haplotype is also a risk factor for MPNs in the Chinese population, and patients with the GG genotype in rs12340895 locus are susceptible to *JAK2* V617F mutation.

Materials and methods

Subjects

The current study was conducted at the Huashan Hospital affiliated to Fudan University, China. Two-hundred and twenty-five Chinese MPN patients aged 14–94 years (mean age 54.3) were included. Among them, 70 had ET, 77 had PV, 36 had PMF, 12 had chronic eosinophilic leukemia, and 30 had chronic myeloid leukemia (CML). Patients were diagnosed according to World Health Organization criteria (2008). Two-hundred and twenty-six healthy volunteers with mean age of 53.5 from the same demographic area were recruited as controls. This study was approved by the Ethics Committee of Huashan Hospital and conducted in accordance with the declaration of Helsinki guidelines for ethics in research. Written informed consent was obtained from each patient prior to collection of the specimens.

Genotyping

Genomic DNA was obtained from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen, Germany). Primers and unlabeled probes were designed by PRIMER 5 software and listed at Table 1. SNP rs12340895, which is in complete linkage disequilibrium with *JAK2* 46/1 haplotype [10], was used as a Tag SNP to examine the *JAK2* 46/1 haplotype. The PCR mix contained the following: 0.5 U Taq HS (Takara, Shiga, Japan), 2 µl 10× PCR Buffer (Mg^{2+} Free, Takara), 1.5 mM $MgCl_2$ (Takara), 0.2 mM dNTP mixture (Takara), 0.05 µM 0895-F, 0.5 µM 0895-R, 0.5 µM 0895-probe, and 0.6 µl 1× SYTO 9 dye (Invitrogen, Carlsbad, California). Melting analysis was performed from 57 to 82 °C with 1 acquisition per 0.5 degree. The typical melting curves of each genotype were presented at Fig. 1. *JAK2* V617F mutation was detected as previously described [11].

Statistical analysis

STATA 10.0 software was used for statistical analysis. Differences in *JAK2* 46/1 haplotype, *JAK2* V617F mutation, age, and gender status between patients and controls were evaluated using the Chi-square test. The association between *JAK2* 46/1 haplotype and risk of MPNs was estimated by odds ratios (ORs) and their 95 % confidence intervals (95 % CIs) using logistic regression. When the number of cases was low ($n < 40$ or $T < 1$), the proportion of 46/1 alleles within each patient subgroup was compared with corresponding controls using Fisher's exact test (2-tailed). According to Jones' results [10], the "Generic Chi-square test' module of the "Java Applets for Power and Sample Size" software (http://www.divms.uiowa.edu/~rlenth/Power/) was used to ensure that the sample size to meet the sample power was >0.80.

Results

Elevated frequency of *JAK2* 46/1 haplotype in MPN patients

High-resolution melting analysis with unlabeled probes has been widely used to genotype SNPs [12, 13]. This is a convenient and reliable method for genotyping and easy

Table 1 Primers and probes for rs12340895 and *JAK2* V617F mutation

	Sequence (5′–3′)	Product length	Modification
0895-F	AGTCTAAAACCAAACTG	102 bp	
0895-R	TGGAAAATAGGGAAT		
0895-probe	CCTTTATTTTAGTGCCTCACAAAGTTTACAT		C3
V617F-F	AGCTTTCTCACAAGCATTTGG	150 bp	
V617F-R	TGACACCTAGCTGTGATCCTG		
V617F-probe	AAATTATGGAGTATGTTTCTGTGGAGACGAGA		C3

The underlined bases are the detected *JAK2* SNP or *JAK2* mutation

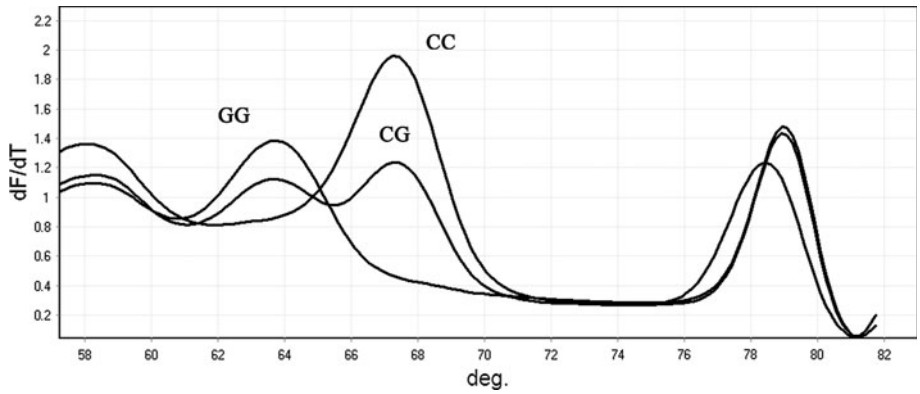

Fig. 1 Typical melting curves of each genotype from rs12340895 locus

Table 2 Frequency of *JAK2* 46/1 haplotype in MPNs patients

	Number of cases	Number of 46/1 alleles	Number of non-46/1 alleles	Frequency 46/1	*P* value	OR (95 % CI)
PV	77	89	65	0.58	6.00E−15	4.88 (3.31–7.20)
ET	70	51	89	0.36	5.59E−04	2.04 (1.36–3.08)
PMF	36	28	44	0.39	1.80E−03	2.27 (1.30–3.95)
CML	30	15	45	0.25	0.588	1.19 (0.64–2.21)
Chronic eosinophilic leukemia	12	6	18	0.25	0.721	1.19 (0.47–2.99)
Controls	226	99	353	0.22	–	–

detection of the *JAK2* 46/1 haplotype that is equal to G allele at rs12340895 locus. We observed three clear melting transitions corresponding to different genotypes, showing unique probe melting patterns with respect to their shape and/or Tm (Fig. 1). For each experiment, we included three positive genotypes that have been confirmed by direct sequencing. In the controls, the *JAK2* 46/1 haplotype frequency was 0.22. The *JAK2* 46/1 haplotype frequencies among the subtypes of MPN patients were significantly different, but all were significantly higher than that in controls. PV patients had the highest frequency of the *JAK2* 46/1 haplotype. The *JAK2* 46/1 haplotype frequency in PV, PMF and ET patients was 0.58 ($P = 6.00\text{E}{-15}$), 0.39 ($P = 1.80\text{E}{-03}$), and 0.36 ($P = 5.59\text{E}{-04}$), respectively (Table 2). However, the *JAK2* 46/1 haplotype frequencies in chronic eosinophilic leukemia (0.25, $P = 0.721$) and Chronic myeloid leukemia (0.25, $P = 0.588$) patients were similar to that of controls.

MPN patients demonstrated increased percentage of GG genotype

To further dissect the role of *JAK2* 46/1 haplotype in MPN patients, we analyzed the rs12340895 genotypes in MPN patients and controls. Due to the similar *JAK2* 46/1 haplotype frequency in CML and chronic eosinophilic leukemia patients to that of controls, the data related to CML and chronic eosinophilic leukemia patients were not analyzed further. The number of MPN patients with CC, CG and GG genotypes was 78 (34.7 %), 105 (46.7 %), and 42 (18.6 %), respectively. The number of controls with CC, CG, and GG genotypes was 132 (58.4 %), 89 (39.4 %), and 5 (2.2 %), respectively (Table 3). There was no significant difference in the distribution of rs12340895 genotypes when grouped by age and gender ($P > 0.05$) (Table 3).

The GG genotype coincided with *JAK2* V617F mutation in PV, ET and PMF patients

JAK2 V617F is considered as a clonal marker for the diagnosis of PV, ET and PMF patients. To further analyze the relationship between the *JAK2* V617F mutation and the *JAK2* 46/1 haplotype, we classified the MPN patients into two groups according to the presence of the *JAK2* V617F mutation. The distribution of rs12340895 genotype in the *JAK2* V617F mutated MPN patients was significantly different to that of controls ($P = 1.67\text{E}{-15}$) (Table 3). The percentage of the GG genotype in controls was 2.2 %;

Table 3 The distribution of rs12340895 genotypes in MPNs patients

Number of genotypes	Mean age (year)	*P* value	Male	Female	*P* value	V617F positive (%)	*P* value	V617F negative (%)	*P* value
MPN (total)									
CC (78)	53	0.919	45	33	0.736	31 (0.40)	1.67E−15	47 (0.60)	0.279
CG (105)	51		55	50	0.399	58 (0.55)		47 (0.45)	
GG (42)	59		19	23	0.880	40 (0.95)		2 (0.05)	
PV									
CC (18)	49	0.913	10	8	0.984	13 (0.72)	4.83E−21	5 (0.28)	1.000
CG (29)	51		21	8	0.178	25 (0.86)		4 (0.14)	
GG (30)	62		14	16	0.945	30 (1.00)		0 (0.00)	
ET									
CC (25)	55	0.783	11	14	0.290	11 (0.44)	2.07E−05	14 (0.56)	0.246
CG (39)	50		18	21	0.199	21 (0.54)		18 (0.46)	
GG (6)	55		2	4	0.567	6 (1.00)		0 (0.00)	
PMF									
CC (12)	60	0.719	8	4	0.649	7 (0.58)	1.99E−04	5 (0.42)	0.269
CG (20)	60		12	8	0.903	12 (0.60)		8 (0.40)	
GG (4)	57		2	2	1.000	4 (1.00)		0 (0.00)	
Control									
CC (132)	52	–	73	59	–	–	–	–	–
CG (89)	56		52	37	–	–		–	
GG (5)	61		3	2	–	–		–	

however, it was 31.0 % in *JAK2* V617F-positive MPN patients. All of the PV, ET and PMF patients with the GG genotype also contained *JAK2* V617F mutation. Among PV, ET and PMF, PV had more significant distribution changes ($P = 4.83E{-}21$) followed by ET ($P = 2.07E{-}05$) and PMF (1.99E−04). In the *JAK2* V617F mutation-negative MPN patients, the distribution of rs12340895 genotypes had no significant difference when compared to controls.

Discussion

JAK2, a member of the Janus family of kinases, is located on chromosome 9p24 and encodes a cytoplasmic tyrosine kinase [14]. It is constitutively associated with the prolactin receptor and involved in a specific subset of cytokine receptor signaling pathways [15, 16]. The *JAK2* V617F mutation is a recently identified somatic mutation that is involved in the majority of MPN diseases [3–6]. Currently, it has been designated a major diagnosis criterion for PV, ET and PMF [7]. Recently, it was reported that *JAK2* V617F-associated disease is highly associated with a specific constitutional *JAK2* haplotype, named the 46/1 haplotype (also called GGCC haplotype) [3, 9, 10]. Tefferi and Vainchenker [7] found that *JAK2* germline genetic variation (46/1 haplotype) affects the disease susceptibility and survival of PMF, regardless of V617F mutational status. In support of this, Pardananai et al. [17] showed that *JAK2* haplotype 46/1 confers susceptibility to develop ET independently of V617F mutation. Furthermore, *JAK2* 46/1 haplotype was shown to be a marker of inappropriate myelomonocytic response to cytokine stimulation, leading to increased risk of inflammation, myeloid neoplasm, and impaired defense against infection [18]. However, most of the investigations if not all were conducted in Caucasian populations. In this study, we found that the *JAK2* 46/1 haplotype is a risk factor for MPNs in the Chinese population, and patients with GG genotype in rs12340895 locus will be more susceptible to *JAK2* V617F mutation.

The rs12340895 SNP and 46/1 haplotype are in complete linkage disequilibrium (LD = 1). Thus, rs12340895 SNP can be used as a tag SNP to test if *JAK2* 46/1 haplotype exists in patients [3, 9, 10]. Our findings in the Chinese population presented here are consistent with early reports in other populations. The *JAK2* 46/1 haplotype frequencies in chronic eosinophilic leukemia (0.25, $P = 0.721$) and CML (0.25, $P = 0.588$) patients are similar to those in controls, suggesting that these two subtypes of MPNs might be not associated with the *JAK2* 46/1 haplotype. The effect of the *JAK2* 46/1 haplotype in PV, PMF and ET patients is dramatic, as the *JAK2* 46/1 haplotype frequencies in PV (0.58, $P = 6.00E{-}15$), PMF (0.39, $P = 1.80E{-}03$), and ET (0.36, $P = 5.59E{-}04$)

patient are significantly higher than that in controls. Taken together, similar to other populations, the *JAK2* 46/1 haplotype is a risk factor in PV, PMF, and ET patients in Chinese population.

The *JAK2* V617F mutation was found to be associated with *JAK2* 46/1 haplotype, but the association and their relationship are largely unknown. We previously demonstrated that the percentage of the *JAK2* V617F mutation in PV, ET and PMF patients is 88.9, 54.5 and 63.6 %, respectively [19]. In the present study, we investigated the relationship between the *JAK2* V617F mutation and *JAK2* 46/1 haplotype and found that the distribution of rs12340895 genotypes in the *JAK2* V617F mutated MPN patients was significantly different to that in controls ($P = 1.67E{-}15$). However, there was no significant difference in the rs12340895 genotypes in the *JAK2* V617F-negative MPN patients and controls ($P = 0.279$). These results suggested that *JAK2* 46/1 haplotype mainly affected *JAK2* V617F mutated MPN patients. The risk factors involved in the *JAK2* V617F-negative MPN patients are still under investigation.

Untreated PV patients homozygous for the *JAK2* 46/1 haplotype showed a progressive increase in the *JAK2* V617F allele burden during the evolution of the disease [20]. MPN patients with high burden of the *JAK2* V617F mutation displayed poor prognosis [21]. The significant association between homozygous carriers of 46/1 haplotype and *JAK2* V617F mutation was not unexpected. The 46/1 haplotype predisposes PV patients to bone marrow fibrosis and affects the survival time of patients with idiopathic myelofibrosis [7]. In later disease stages MPN patients could develop acute myeloid leukemia (AML). Patients with the 46/1 haplotype have a higher possibility of this transformation and infection, and will eventually die. Thus, the *JAK2* 46/1 haplotype is helpful not only for the screening of MPN, but also for the prediction of the severity of the disease and outcome of the patients. Unfortunately, in the current study, we were unable to get the patient's information before and after treatment, thus could not recapitulate these findings. In the future, we will increase the sample size to investigate the association between *JAK2* 46/1 haplotype and prognosis of the MPN patients.

In this study of Chinese MPN patients, we found that almost all patients (95 %) with the rs12340895 GG genotype showed *JAK2* V617F mutation, especially in PV, ET and PMF patients (100 %). The percentage of the *JAK2* V617F mutation in the GG genotype of MPN patients was significantly higher than in CG (55 %) or CC (40 %) genotypes. In contrast, Andrikovics et al. [22] demonstrated that only 90 % GG genotype patients had the V617F mutation. Other groups reported that the percentage of the V617F mutation in GG genotype MPN patients was around 77.8–82.8 % [7, 23]. These discrepancies might be due to the differences in the size or ethnics descent of the patient cohorts analyzed. Therefore, studies with a larger number of Chinese patients should be conducted to clarify the association between the *JAK2* V617F mutation and the *JAK2* 46/1 haplotype.

In conclusion, we reported that the *JAK2* 46/1 haplotype is a risk factor for MPNs in Chinese population and patients with GG genotype in the rs12340895 locus will more frequently have the *JAK2* V617F mutation when compared to other genotypes.

Acknowledgments This study was supported by a grant from the National Clinical Key Subject, and two grants from the Shanghai Science and Technology Commission (Grant No. 11JC1401800 and No. 10411950200) and Scientific Research Foundation for New teacher of Fudan University (JJF151002).

Conflict of interest The authors declare that they have no conflict of interests.

References

1. Abdel-Wahab O. Genetics of the myeloproliferative neoplasms. Curr Opin Hematol. 2011;18(2):117–23.
2. Ohyashiki JH, Yoneta M, Hisatomi H, Iwabuchi T, Umezu T, Ohyashiki K. The C allele of JAK2 rs4495487 is an additional candidate locus that contributes to myeloproliferative neoplasm predisposition in the Japanese population. BMC Med Genet. 2012;13:6.
3. Olcaydu D, Harutyunyan A, Jager R, Berg T, Gisslinger B, Pabinger I, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. Nat Genet. 2009;41(4):450–4.
4. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005;434(7037):1144–8.
5. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;7(4):387–97.
6. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005;365(9464):1054–61.
7. Tefferi A, Vainchenker W. Myeloproliferative neoplasms: molecular pathophysiology, essential clinical understanding, and treatment strategies. J Clin Oncol. 2011;29(5):573–82.
8. Landgren O, Goldin LR, Kristinsson SY, Helgadottir EA, Samuelsson J, Bjorkholm M. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24,577 first-degree relatives of 11,039 patients with myeloproliferative neoplasms in Sweden. Blood. 2008;112(6):2199–204.
9. Kilpivaara O, Mukherjee S, Schram AM, Wadleigh M, Mullally A, Ebert BL, et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. Nat Genet. 2009;41(4):455–9.
10. Jones AV, Chase A, Silver RT, Oscier D, Zoi K, Wang YL, et al. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. Nat Genet. 2009;41(4):446–9.

11. Wu Z, Yuan H, Zhang X, Liu W, Xu J, Zhang W, et al. Development and inter-laboratory validation of unlabeled probe melting curve analysis for detection of JAK2 V617F mutation in polycythemia vera. PLoS ONE. 2011;6(10):e26534.
12. Vossen RH, van Duijn M, Daha MR, den Dunnen JT, Roos A. High-throughput genotyping of mannose-binding lectin variants using high-resolution DNA-melting analysis. Hum Mutat. 2010;31(4):E1286–93.
13. Liu SM, Xu FX, Shen F, Xie Y. Rapid genotyping of APOA5 -1131T>C polymorphism using high resolution melting analysis with unlabeled probes. Gene. 2012;498(2):276–9.
14. Bole-Feysot C, Goffin V, Edery M, Binart N, Kelly PA. Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr Rev. 1998;19(3):225–68.
15. Reddy MM, Deshpande A, Sattler M. Targeting JAK2 in the therapy of myeloproliferative neoplasms. Expert Opin Ther Targets. 2012;16(3):313–24.
16. Ghoreschi K, Laurence A, O'Shea JJ. Janus kinases in immune cell signaling. Immunol Rev. 2009;228(1):273–87.
17. Pardanani A, Lasho TL, Finke CM, Gangat N, Wolanskyj AP, Hanson CA, et al. The JAK2 46/1 haplotype confers susceptibility to essential thrombocythemia regardless of JAK2V617F mutational status-clinical correlates in a study of 226 consecutive patients. Leukemia. 2010;24(1):110–4.
18. Hermouet S, Vilaine M. The JAK2 46/1 haplotype: a marker of inappropriate myelomonocytic response to cytokine stimulation, leading to increased risk of inflammation, myeloid neoplasm, and impaired defense against infection? Haematologica. 2011;96(11):1575–9.
19. Zhang X, Gu X, Liu S, Ma W, Guan M. Detection of JAK2 V617F mutation by real-time PCR. Clin J Lab Med (Chinese). 2009;32(5):583–6.
20. Alvarez-Larran A, Angona A, Martinez-Aviles L, Bellosillo B, Besses C. Influence of JAK2 46/1 haplotype in the natural evolution of JAK2V617F allele burden in patients with myeloproliferative neoplasms. Leuk Res. 2012;36(3):324–6.
21. Vannucchi AM, Antonioli E, Guglielmelli P, Longo G, Pancrazzi A, Ponziani V, et al. Prospective identification of high-risk polycythemia vera patients based on JAK2(V617F) allele burden. Leukemia. 2007;21(9):1952–9.
22. Andrikovics H, Nahajevszky S, Koszarska M, Meggyesi N, Bors A, Halm G, et al. JAK2 46/1 haplotype analysis in myeloproliferative neoplasms and acute myeloid leukemia. Leukemia. 2010;24(10):1809–13.
23. Guglielmelli P, Biamonte F, Spolverini A, Pieri L, Isgro A, Antonioli E, et al. Frequency and clinical correlates of JAK2 46/1 (GGCC) haplotype in primary myelofibrosis. Leukemia. 2010;24(8):1533–7.