A risk of essential thrombocythemia in carriers of constitutional *CHEK2* gene mutations

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ABSTRACT

Germline mutations of the *CHEK2* gene have been reported in some myeloid and lymphoid malignancies, but their impact on development of essential thrombocythemia has not been studied. In 16 out of 106 (15.1%) consecutive patients, newly diagnosed with essential thrombocythemia, we found one of four analyzed *CHEK2* mutations: I157T, 1100delC, IVS2+1G>A or del5395. They were associated with the increased risk of disease (OR=3.8; *P*=0.002). The median age at ET diagnosis among *CHEK2+/JAK2V617F+* patients was seven years lower than that among *CHEK2-/JAK2V617F+* (52 *vs*. 59 years; *P*=0.04), whereas there was no difference in the medians of hematologic parameters between these groups. The results obtained suggest that *CHEK2* mutations could potentially contribute to the susceptibility to essential thrombocythemia. The germline inactivation of *CHEK2*, as it seems, has no direct impact on the development of disease, but it could cause disruption of cell cycle checkpoints and initiate or support the cancerogenic process of essential thrombocythemia at a younger age.

Key words: essential thrombocythemia, congenital *CHEK2* mutations.

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Introduction

The genetic background of essential thrombocythemia (ET) is not yet well known. Sporadic cases of ET are those most frequently observed, but patients with ET family history have also been reported, suggesting a hereditary nature of the disease.[1,2] Various reports on the association between acquired and inherited gene mutations and risk of ET have been presented, but the impact of constitutional mutations of tumor susceptibility genes, e.g. *CHEK2*, on the disease development has not been studied to date.

CHEK2 is the tumor suppressor gene that protects cells against too rapid, uncontrolled growth by regulating of cell division. It encodes cell cycle G2 checkpoint kinase, which is a key mediator in cellular response to various types of DNA damage. The CHEK2 kinase is activated on *ATM*-dependent pathways and then phosphorylates substrates, such as p53, BRCA1, CDC25A and CDC25C, involved in the cell cycle checkpoint control through coordination of DNA repair, cell cycle progression and apoptosis.[3]

Germline *CHEK2* mutations (i.e. 1100delC, I157T) were found in Li-Fraumeni syndrome,[4] some lymphoid malignancies,[5-7] and also in myeloid malignancies, e.g. myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), with a low frequency.[6,8] Collado *et al.*[9] described lack of *CHEK2* germline mutations in Spanish patients with AML. However, the author noted that association of these mutations with cancer risk depends mainly on their incidence in the general population. Lack of a mutation, or its very low frequency in the control and study groups, make the presence of this mutation clinically irrelevant.[9]

Constitutional mutations of *CHEK2* were reported in many types of solid tumors, including breast, ovary, colon, prostate and thyroid gland cancers. It was estimated that *CHEK2* mutations cause low to moderate genetic risk of the development of solid tumors.[10-14]

In the general Polish population, the most common are the I157T missense mutation (4.8% frequency) and three premature protein-truncating mutations: IVS2+1G>A, del5395 and 1100delC (0.4%, 0.4% and 0.2% frequency, respectively).[15] The splice-site mutation IVS2+1G>A results from nucleotide transition G→A and 4-bp insertion in the mutant transcript, due to an abnormal splicing between exons 2 and 3. The 5,395-bp deletion includes exon 9 and 10.[16]

In this report, we present the results of our research on association between the above mentioned four *CHEK2* muta-

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tions and a risk of ET. The relationship between a congenital *CHEK2* mutation and the acquired V617F mutation of the Janus tyrosine kinase 2 gene (*JAK2*), the important marker of myeloproliferative neoplasms,[17] was also investigated, as well as its relation to patients' age, hematologic features at ET diagnosis, as well as family history of cancer.

Design and Methods

Patients

A total of 106 consecutive patients, newly diagnosed with ET according to WHO 2008 criteria[18] in two Polish hematology centers (Wrocław and Toruń) were involved in the study. The median age at ET diagnosis was 56.5 years (≈57 years; range 20-85). Data from medical records were collected for each patient: hemoglobin and hematocrit level, white blood cell (WBC) count and platelet (PLT) count at ET diagnosis.

In families with suspicion of a congenital *CHEK2* mutation, molecular tests were performed (8 out of 16 invited families agreed to testing; a total of 22 subjects).

Fifty-nine percent of ET patients originated from families with at least one case of cancer diagnosed in a close relative. The most frequent were breast, lung, colon, stomach, pancreas, prostate and ovary cancer, as well as acute or chronic leukemia (in 6 families) or ET (in 4 families). One family fulfilled Li-Fraumeni-like syndrome criteria.[19] In families of 4 patients (diagnosed with ET at the ages of 34, 62, 61 and 62 years) another ET case was diagnosed in the daughter at the age of 14, the mother at the age of 53, the sister at the age of 56, and the brother at the age of 60, respectively. Forty-three out of 106 (41%) ET patients originated from families with no other cancer case.

Medical records confirmed the ET diagnosis and the clinical history of all patients. The control group consisted of 200 healthy persons from the Wrocław and Toruń regions. The data on the general Polish population published by Cybulski *et al.*[15] were used as the second control group. Informed consent was obtained from all study subjects. The study was approved by the Ethics Committee of the Collegium Medicum, Nicolaus Copernicus University in Bydgoszcz, Poland.

Molecular analysis

The *CHEK2* mutations were investigated in DNA from peripheral blood leukocytes, extracted by standard salting-out method. The constitutional character of mutations was verified in mutation-positive patients by analysis of DNA from buccal swabs, extracted using Swab-Extract DNA Purification Kit (EURx, Poland). The I157T and IVS2+1G>A were examined by RFLP-PCR,[20] the 1100delC by ASO-PCR,[20] and the del5395 by multiplex-PCR with two primer pairs flanking breakpoint sites in introns 8 and 10.[15] Mutation-positive cases were confirmed by sequencing analysis using ABI-PRISM 3130 Genetic Analyzer (Applied Biosystems, USA). The *JAK2* V617F was detected in DNA from peripheral blood and from buccal swabs by allele-specific PCR described by Jones *et al.*,[21] and cases with suspicion of homozygosity were examined by sequencing analysis.

Statistical analysis

Statistical analysis included a comparison of the prevalence of variant alleles in the studied and control groups, calculation of odds ratios (ORs) from two-by-two tables, and calculation of statistical significance of differences between tested groups using the Fisher's exact test. Moreover, values of median and arithmetic mean (and standard deviation) of the age and hematologic parameters at ET diagnosis in groups of patients were calculated and compared. All calculations were performed using the Interactive Statistical Calculation Pages program for Windows.

Results and Discussion

Analysis of CHEK2 mutations

A *CHEK2* mutation was found in 16 out of 106 ET patients (15.1%). All mutations detected in DNA from peripheral blood were also found in DNA from buccal swabs; this confirmed their constitutional character. They were significantly more frequent in the group of ET patients than in both control groups and were associated with the increased ET risk (our controls: 4.5%; OR=3.8; P=0.002; the general Polish population 5.8%; OR=2.9; P=0.0005). The elevated risk of ET also was shown in subgroups of patients: with IVS2+1G>A only, with protein-truncating mutations together, and with I157T only (Table 1). The ET risk for carriers of congenital *CHEK2* mutations was comparable with their risk of breast or prostate cancer.[12,15]

Among patients with ET diagnosed under the age of 57 (the median age at ET diagnosis), the prevalence of *CHEK2* mutations (except IVS2+1G>A) was significantly higher than among patients diagnosed with ET at/above this age compared to both controls. The IVS2+1G>A frequency was statistically higher in the group of ET patients with ET diagnosed at an older age compared to the general Polish population (Table 1).

The I157T mutation occurs in exon 3 within the highly conserved protein-interaction domain FHA (forkhead homology-associated), basic for the *CHEK2* activation after DNA damage. As functional studies revealed, CHEK2 protein with the I157T alteration can not bind to and phosphorylate the downstream targets, CDC25A and p53, in phosphorylation cascades of the cell cycle. This might disrupt the regulatory function of the protein thus contributing to transformation and uncontrolled proliferation of affected cells.[3]

In our analysis, three frameshift mutations resulting in premature protein-truncation also led to instability of the protein and a decrease or loss of *CHEK2* activity. The IVS2+1G>A eliminates part of FHA domain and the entire kinase activation domain of CHEK2 protein, fundamental for interactions with other proteins in response to DNA damage. Similarly, 1100delC and del5395 mutations result in elimination of the kinase activation domain.[7,16,22] In our study, a sequence analysis of all *CHEK2* mutation-positive cases confirmed their heterozygous state. Heterozygosity of mutant *CHEK2* gene may cause a decrease in the CHEK2 protein concentration below the critical level and may lead to a reduction or loss of gene activity.[3]

The median age at ET diagnosis in 16 *CHEK2* mutation carriers was four years lower than the median calculated for non-carriers, but this difference was not statistically significant (53 *vs.* 57 years; P=0.14). We also showed no correlation of *CHEK2* mutations with any hematologic parameters, the basis for a correct diagnosis of ET (Table 2).

The investigation of 8 families confirmed the hereditary character of mutations found in ET patients by their detection in first and second degree relatives (Figure 1 A-H). Out of 16 patients with a *CHEK2* mutation, 7 (44%) originated from families with organ cancers (Figure 1A-D). In the family with LFL syndrome, I157T was found in the ET patient, in his healthy mother and her sister's healthy son

(a sister died from breast cancer which had been diagnosed at the age of 37). However, in the patient's healthy father, and in a brother with childhood ALL, the I157T was not found (Figure 1C). However, among the four families with 2 cases of ET a hereditary *CHEK2* mutation was shown in only one. In this family, I157T was found in 3 subjects from three successive generations, among whom 2 were diagnosed with ET (Figure 1D). Nine (21%) of *CHEK2*-positive ET patients originated from families with no other cancer case (4 of the families are presented in Figure 1E-H). These results suggest that *CHEK2* mutations are rather associated with sporadic ET than with the familial form, although to confirm this observation a larger group of sporadic and familial ET cases should be tested.

Analysis of the JAK2 V617F

The V617F mutation was found in 61 (58%) patients, one of whom was homozygous. However, in the group of patients with ET diagnosed under the age of 57, the frequency of V617F was much lower (49%) than in the group with ET diagnosed at or above this age (66%) (Table 2). The V617F was not found in DNA from buccal swabs of ET patients, in DNA from peripheral blood of our controls, and in all but one of patients' relatives (from 8 families). V617F was found in the daughter (diagnosed with ET at the age of 14) of the woman (diagnosed with ET at the age of 34) (Figure 1D). The homozygosity of V617F was found in a patient with *CHEK2* I157T (age at ET onset 50 years) whose family did not consent to molecular studies (Table 2).

In the group of ET patients with V617F compared to the group without it, median hemoglobin and hematocrit level, as well as the WBC count at disease diagnosis were significantly higher, whereas median of the PLT count was significantly lower, findings confirmed by many authors. Moreover, the median age at ET diagnosis among V617F positive patients was five years higher than among V617F negative patients, but this difference was not statistically important (54 *vs.* 59 years; P=0.19) (Table 2).

Table 1. The association between a constitutional *CHEK2* mutation and the risk of essential thrombocythemia.

A *CHEK2* mutation and groups of patients	Carriers/total N.	%	OR	95% CI	Controls[a] n=200 *P* value	OR	Controls[b] n=5,496 95% CI	*P* value
IVS2 + 1G>A								
Under the age of 57	1/53	1.9	3.8	0.2-62.2	0.38	4.8	0.6-36.2	0.20
At/over the age of 57	2/53	3.7	7.8	0.7-87.8	0.11	9.7	2.2-42.6	0.02[c]
All cases	3/106	2.8	5.8	0.6-56.4	0.12	7.2	2.1-24.6	0.01[c]
Controls[a]	1/200	0.5	1.0					
Controls[b]	22/5,496	0.4	1.0					
1100delC								
Under the age of 57	1/53	1.9	-	-	-	8.8	1.1-68.8	0.12
At/over the age of 57	-	-	-	-	-	-	-	-
All cases	1/106	0.9	-	-	-	4.3	0.6-33.8	0.22
Controls[a]	0/200	0	-					
Controls[b]	12/5,496	0.2	1.0					
del5395								
Under the age of 57	2/53	3.7	7.8	0.7-87.8	0.11	8.9	2.1-38.8	0.02[c]
At/over the age of 5	-	-	-	-	-	-	-	-
All cases	2/106	1.9	3.8	0.3-42.7	0.28	4.4	1.0-18.8	0.09
Controls[a]	1/200	0.5	1.0					
Controls[b]	24/5,496	0.4	1.0					
Any protein truncating								
Under the age of 57	4/53	7.6	8.0	1.4-45.4	0.02[c]	7.6	2.7-21.9	0.003[c]
At/over the age of 57	2/53	3.7	3.9	0.5-28.2	0.19	3.7	0.9-15.5	0.11
All cases	6/106	5.7	5.9	1.2-29.9	0.02[c]	5.6	2.4-13.3	0.001[c]
Controls[a]	2/200	1.0	1.0					
Controls[b]	58/5,496	1.1	1.0					
I157T								
Under the age of 57	6/53	11.3	3.5	1.1-11.0	0.03[c]	2.5	1.1-6.0	0.04[c]
At/over the age of 57	4/53	7.6	2.2	0.6-8.0	0.25	1.6	0.6-4.5	0.32
All cases	10/106	9.4	2.8	1.1-7.8	0.04[c]	2.1	1.1-4.0	0.04[c]
Controls[a]	7/200	3.5	1.0					
Controls[b]	264/5,496	4.8	1.0					
Total *CHEK2* mutations								
Under the age of 57	10/53	18.9	4.9	1.9-12.9	0.001[c]	3.7	1.9-7.5	0.001[c]
At/over the age of 57	6/53	11.3	2.7	0.9-8.0	0.09	2.1	0.9-4.8	0.13
All cases	16/106	15.1	3.8	1.6-8.9	0.002[c]	2.9	1.7-4.9	0.0005[c]
Controls[a]	9/200	4.5	1.0					
Controls[b]	321/5,49	5.8	1.0					

[a]*Controls consisting of healthy persons from the Wrocław and Toruń regions of Poland.* [b]*Controls of the general Polish population, published by Cybulski* et al.[15] [c]*Statistically important.*

Table 2. The age and hematologic features at ET diagnosis in the groups of patients with or without *CHEK2* mutations (*CHEK2+* or *CHEK2-*) and with or without the *JAK2* V617F mutation (*JAK2+* or *JAK2-*).

	CHEK2 mutations			JAK2 V617F				CHEK2 mutations / JAK2 V617F		
	CHEK2-	*CHEK2+*	*P* value	*JAK2-*	*JAK2+*	*P* value	Homozygotes	*CHEK2-* *JAK2+*	*CHEK2+* JAK2+	*P* value
Patients										
Under the age of 57 (n=53)	43 (81.1%)	10 (18.9%)		27 (51.0%)	26 (49.0%)		1 (1.9%)	20 (37.7%)	6 (11.3%)	
At/over the age of 57 (n=53)	47 (88.7%)	6 (11.3%)		18 (34.0%)	35 (66.0%)		-	32 (60.3%)	3 (5.7%)	
All patients (n=106)	90 (84.9%)	16 (15.1%)		45 (42.5%)	61 (57.5%)		1 (0.9%)	52 (49.0%)	9 (8.5%)	
Male/female	30/60	6/10		15/30	21/40		0/1	17/35	4/5	
Age of all patients (years)[a]	57 (20-85)	53 (21-72)	0.14	54 (20-85)	59 (21-85)	0.19	50	59 (23-85)	52 (21-68)	0.04[b]
Hematologic parameters										
Hematocrit (%)[a]	43.2 (29.4-58.0)	42.1 (34.9-51.5)	0.76	41.8 (29.4-53.2)	44.2 (30.1-58.0)	0.02[b]	34.9	44.4 (30.1-58)	43.7 (34.9-51.5)	0.51
Hemoglobin (g/dL)[a]	13.9 (9.3-19.0)	14.1 (12.2-16.9)	0.64	13.5 (10.9-16.5)	14.4 (9.3-19.0)	0.003[b]	15.3	14.2 (9.3-16.9)	15.3 (13.3-16.9)	0.17
WBC (x10^9/L)[a]	9.8 (4.4-21.3)	10.0 (5.8-17.2)	NaN	9.3 ((5.7-17.4)	10.2 (4.4-21.3)	0.05[b]	17.2	10.2 (4.42-21.3)	9.6 (7.0-17.2)	0.72
PLT (x10^9/L)[a]	886 (472-2179)	904 (493-1582)	0.88	998 (492-2179)	834 (472-2146)	0.001[b]	960	799 (472-2146)	854 (503-1297)	0.77

[a]*Median value (range);* [b]*statistically important.*

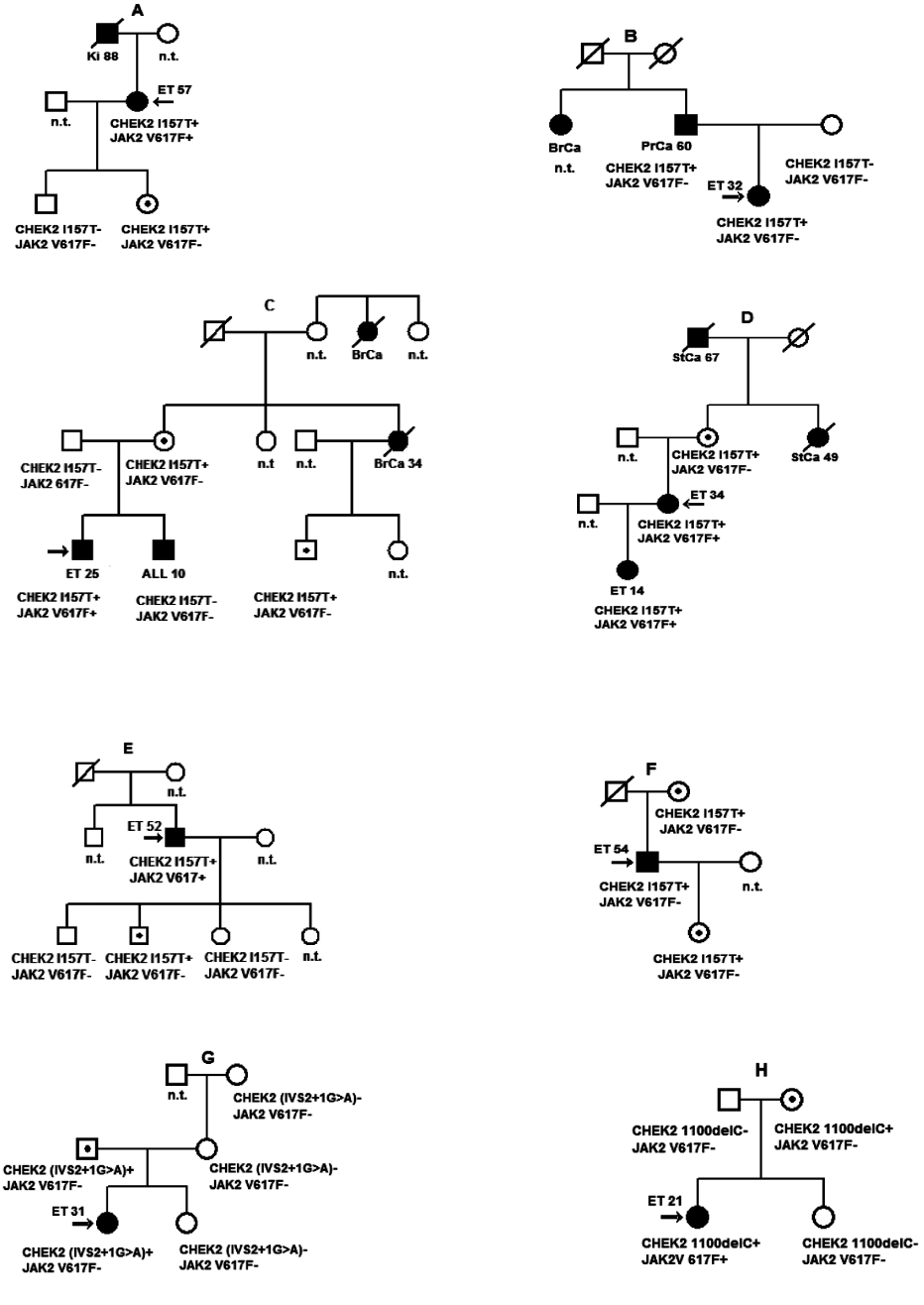

Figure 1. (A-F) Families with *CHEK2* I157T mutation. (G) The family with *CHEK2* IVS2+1G>A mutation. (H) The family with *CHEK2* 1100delC mutation. Black symbols designate persons affected with cancer. The age of cancer onset is given after a disease symbol or above an arrow indicating a proband. ET: essential thrombocythemia; ALL: acute lymphoblastic leukemia; Br: breast cancer; St: stomach cancer; Pr: prostate cancer; Ki: kidney cancer; n.t.: not tested. The presence of a mutation is designated +, absence -.

Correlation of CHEK2 mutations (CHEK2+) with the JAK2 V617F (JAK2+)

The acquired *JAK2* V617F mutation was found in 9 of 16 (56%) ET patients, carriers of congenital *CHEK2* mutation. In 6 of 9 (67%) *CHEK2+/JAK2+* patients, ET was diagnosed under the age of 57.

Medians of hematologic parameters at ET diagnosis in the groups of *CHEK2+/JAK2+* and *CHEK2-/JAK2+* patients were similar. However, the median age at ET diagnosis among *CHEK2+/JAK2+* patients was seven years lower than among *CHEK2-/JAK2+* patients (52 *vs.* 59 years; *P*=0.04) (Table 2).

These results suggest that in hereditarily predisposed individuals, with a dysfunction of the tumor suppressor *CHEK2* gene which performs a key role in regulation of the cell cycle, easier and faster acquisition of *JAK2* V617F mutation may occur, leading to clinical consequences at a younger age. However, it should be noted that the V617F was found in only 9 of 16 ET patients with a *CHEK2* mutation. Therefore, further studies should be carried out to verify whether in *CHEK2+/JAK2V617F-* patients other mutations of *JAK2* are present. They should also include an analysis of *JAK2* 46/1 haplotype, which increases the risk of ET regardless of the acquisition of V617F,[23] and its relation with *CHEK2* mutations.

On the basis of the results obtained, we can conclude that *CHEK2* gene mutations could potentially contribute to the susceptibility to ET. It seems that the germline inactivation of *CHEK2* has no direct impact on the development of ET, but it can cause disruption of cell cycle checkpoints and initiate or support the ET cancerogenic process at a younger age.

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References

1. Landgren O, Goldin LR, Kristinsson SY, Helgadottir EA, Samuelsson J, Björkholm 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.
2. Cazzola M. Molecular basis of thrombocytosis. Haematologica. 2008;93(5):646-8.
3. Bartek J, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell. 2003;3(5):421-9.
4. Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, et al. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science. 1999; 286(5449):2528-31.
5. Rudd MF, Sellick GS, Webb EL, Catovsky D, Houlston RS. Variants in the ATM-BRCA2-CHEK2 axis predispose to chronic lymphocytic leukemia. Blood. 2006;108 (2):638-44.
6. Hangaishi A, Ogawa S, Qiao Y, Wang L, Hosoya N, Yuji K, et al. Mutations of Chk2 in primary hematopoietic neoplasms. Blood. 2002;99(8):3075-7.
7. Sellick GS, Sullivan K, Catovsky D, Houlston RS. CHEK2*1100delC and risk of chronic lymphocytic leukemia. Leuk Lymphoma. 2006;47(12):2659-60.
8. Aktas D, Arno MJ, Rassool F, Mufti GJ. Analysis of CHK2 in patients with myelodysplastic syndromes. Leuk Res. 2002;26(11):985-7.
9. Collado M, Landt O, Barragán E, Lass U, Cervera J, Sanz MA, Bolufer P. Locked nucleic acid-enhanced detection of 1100delC*CHEK2 germ-line mutation in Spanish patients with hematologic malignancies. Clin Chem. 2004;50(11):2201-4.
10. Cybulski C, Górski B, Huzarski T, Masojć B, Mierzejewski M, Debniak T, et al. CHEK2 is a multiorgan cancer susceptibility gene. Am J Hum Genet. 2004;75(6):1131-5.
11. Seppälä EH, Ikonen T, Mononen N, Autio V, Rökman A, Matikainen MP, et al. CHEK2 variants associate with hereditary prostate cancer. Br J Cancer. 2003;89(10):1966-70.
12. Cybulski C, Wokołorczyk D, Huzarski T, Byrski T, Gronwald J, Górski B, et al. A deletion in CHEK2 of 5,395 bp predisposes to breast cancer in Poland. Breast Cancer Res Treat. 2007;102(1):119-22.
13. Dębniak T, Cybulski C, Kurzawski G, Górski B, Huzarski T, Byrski T, et al. Low-risk genes and multi-organ cancer risk in Polish population. Hered Cancer Clin Pract. 2006;4(1):52-5.
14. Nusbaum R, Vogel KJ, Ready K. Susceptibility to breast cancer: hereditary syndromes and low penetrance genes. Breast Dis 2006-2007;27:21-50.
15. Cybulski C, Wokolorczyk D, Huzarski T, Byrski T, Gronwald J, Górski B, et al. A large germline deletion in the Chek2 kinase gene is associated with an increased risk of prostate cancer. J Med Genet. 2006;43(11): 863-6.
16. Dong X, Wang L, Taniguchi K, Wang X, Cunningham JM, McDonnell SK, et al. Mutations in CHEK2 associated with prostate cancer risk. Am J Hum Genet. 2003;72(2):270-80.
17. Passamonti F, Rumi E. Clinical relevance of JAK2 (V617F) mutant allele burden. Haematologica. 2009;94(1):7-10.
18. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. Leukemia. 2008;22(1):14-22.
19. Eeles RA. Germline mutations in the TP53 gene. Cancer Surv 1995;25:101-24.
20. Cybulski C, Huzarski T, Górski B, Masojć B, Mierzejewski M, Debniak T, et al. A novel founder CHEK2 mutation is associated with increased prostate cancer risk. Cancer Res. 2004;64(8):2677-9.
21. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood. 2005; 106(6):2162-8.
22. Li J, Williams BL, Haire LF, Goldberg M, Wilker E, Durocher D, et al. Structural and functional versatility of the FHA domain in DNA-damage signaling by the tumor suppressor kinase Chk2. Mol Cell. 2002;9(5): 1045-54.
23. Pardanani A, Lasho TL, Finke CM, Gangat N, Wolanskyj AP, Hanson CA, Tefferi A. 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.