doi:10.4149/neo_2011_05_392

Alterations of *CHEK2* forkhead-associated domain increase the risk of Hodgkin lymphoma

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Received January 31, 2011

Checkpoint kinase 2 gene (*CHEK2*) codes for an important mediator of DNA damage response pathway. Mutations in the *CHEK2* gene increase the risk of several cancer types, however, their role in Hodgkin lymphoma (HL) has not been studied so far. The most frequent *CHEK2* alterations (including c.470T>C; p.I157T) cluster into the forkhead-associated (FHA) domain-coding region of the *CHEK2* gene. We performed mutation analysis of the *CHEK2* gene segment coding for FHA domain using denaturing high-performance liquid chromatography in 298 HL patients and analyzed the impact of characterized *CHEK2* gene variants on the risk of HL development and progression-free survival (PFS). The overall frequency of *CHEK2* alterations was significantly higher in HL patients (17/298; 5.7%) compared to the previously analyzed non-cancer controls (19/683; 2.8%; p = 0.04). Presence of any alteration within the analyzed region of the *CHEK2* gene was associated with increased risk of HL development (OR = 2.11; 95% CI = 1.08 – 4.13; p = 0.04). The most frequent I157T mutation was found in 4.0% of HL patients and 2.5% of controls (p = 0.22), however, the frequency of 5 other alterations (excluding I157T) was significantly higher in HL cases and associated with increased risk of HL development (OR = 5.81; 95% CI = 1.12 – 30.12; p = 0.03). PFS in HL patients did not differ between *CHEK2* mutation carriers and non-carriers. The predominant I157T mutation together with other alterations in its proximity represent moderate genetic predisposition factor increasing the risk of HL development.

Key words: Hodgkin lymphoma; checkpoint kinase 2 gene (CHEK2, CHK2); germ-line mutation; genetic predisposition; risk assessment

Hodgkin lymphoma (HL) is a malignant disease histologically characterized by the presence of large Hodgkin and Reed-Sternberg cells derived from B lymphocytes that constitute a minority of the cell population in affected lymphatic nodes. The annual incidence of HL in Europe is approximately 2.5 cases per 100 000 inhabitants [1]. Besides known environmental and life style risk factors (such as EBV and HIV infection, immunodeficiency or socioeconomic status), risk of HL development is modified by genetic background [2]. This hypothesis is supported by an increased incidence of HL reported in monozygotic twins [3] and first degree relatives of lymphoma patients [4, 5]. The CHK2 protein (coded by *CHEK2* gene, OMIM 604373) is a member of ATM-CHK2-p53 signaling pathway activated upon recognition of DNA double-strand breaks (DSB). CHK2 is responsible for transmission and amplification of the signal from activated ATM kinase to the effector proteins involved in DNA repair, cell cycle arrest and apoptosis [6]. The CHK2 protein contains the N-terminal SQ/TQ cluster domain, the central forkhead-associated (FHA) domain and the C- terminal serine/threonine kinase domain [7]. CHK2 activation is initiated by ATM kinase-mediated phosphorylation of Thr68 that induces homodimerization of CHK2 monomers (via their FHA domains) and consequent autophosphorylation of their kinase domains [8, 9]. It has been shown that mutation of Thr68 or alterations of FHA domain impairs CHK2 dimerization and its activation [10, 11].

The *CHEK2* gene has been considered a multiorgan cancer susceptibility gene predisposing to the development of breast, colon, kidney, prostate, and thyroid cancers [12]. The vast majority of *CHEK2* mutations contributing to cancer predisposition are clustered within the fragment coding for FHA domain. The role of *CHEK2* alterations as a risk factor of HL has never been evaluated; therefore, we performed mutation analysis in the region coding for FHA domain of CHK2 in 298 HL patients and analyzed the

impact of characterized *CHEK2* gene variants on the risk of HL development and on progression-free survival (PFS).

Materials and methods

Study population. Two hundred and ninety-eight patients with histologically confirmed diagnosis of HL treated with first-line treatment between the years 2005 and 2010 at three Prague's hematological departments were enrolled to this study. Clinical characteristics of patients are summarized in Table 1. Control group of 683 non-cancer individuals was described in detail previously including the results of *CHEK2* mutation analysis [13, 14]. All lymphoma patients and controls were of Caucasian origin from the same geographical area of the Czech Republic. All participating subjects signed an informed consent with genetic testing approved by local ethical committees.

Mutation analysis. Genomic DNA was isolated from whole peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen) or using automated DNA preparation system (MagNA Pure LC 2.0, Roche) according to the manufacturer's instructions. Mutation analysis of FHA-coding region was performed as described previously [13]. Briefly, FHA-coding region of *CHEK2* gene (covering exon 2 and 3) was PCR-amplified in a single fragment and analyzed by denaturing high-performance liquid chromatography (DHPLC, WAVE system, Transgenomic). Samples with aberrant elution profiles on DHPLC were re-amplified and bi-directionally sequenced using ABI 3130 (Applied Biosystemes). Web-based program Align GVGD (http://agvgd.iarc.fr/) was used to predict functional relevance of found *CHEK2* missense variants [15, 16].

Statistical analysis. The two-sided Fisher's exact test was used for the evaluation of differences in alteration frequencies between analyzed groups. Crude odds ratios (OR) were calculated from 2 x 2 contingency tables using unconditional Mantel–Haenszel statistics, differences in clinical characteristics between alteration carriers and non-carriers using Chi-square test and nonparametric ANOVA. Analysis of PFS was performed by Kaplan-Meier method in a subgroup of patients (N = 215) that i) were enrolled to the study at the time of diagnosis and ii) in which the survival data were available. Differences of survival curves were evaluated by Wilcoxon and Log-rank tests. PFS was defined as the interval from the date of diagnosis to the date of progression, relapse or death from any cause or last follow-up date after the first-line treatment. The median follow-up of patients was 22.4 months. All analyses were performed using NCSS 2007 statistical program (NCSS).

Table 1. Clinical characteristics of HL patients (n = 298)

Gender N (%)	
Male	150 (50.3)
Female	148 (49.7)
Age at diagnosis median of years (range)	32.2 (14.0-83.7)
Histological subtype N (%)	
NLPHL	14 (4.7)
NS	199 (66.8)
MC	69 (23.2)
Other	16 (5.4)
Clinical stage N (% of known)	
I	19 (6.5)
II	140 (48.1)
III	62 (21.3)
IV	70 (24.3)
Unknown	7

NLPHL – Nodular lymphocyte predominant Hodgkin lymphoma; *NS* – Nodular sclerosis classical Hodgkin lymphoma; *MC* – Mixed cellularity classical Hodgkin lymphoma

Results and discussion

To evaluate the risk of HL development, the mutation analysis of *CHEK2* FHA-coding region was performed in 298 samples of HL patients. We ascertained six different *CHEK2* alterations localized within FHA-coding region (c.470T>C, c.475T>C, c.542G>A) or in its proximity (IVS1-5T>A, IVS2+24C>T, IVS2-54C>T (this variant was erroneously referred to as IVS2-55C>T in our previous publications [14,17]; Table 2). The overall fre-

Table 2. Frequencies of alterations identified in *CHEK2* FHA-coding region

Exon/ intron	Alteration	HL patients N (%)	Controls N (%)	OR [a]	95% CI [a]	*p* value [a]
-	None	281 (94.3)	664 (97.2)	Reference (1.00)		
e3	c.470T>C (p.I157T)	12 (4.0)	17 (2.5)	1.64	0.78-3.49	0.22
e3	c.475T>C (p.Y159H)	1 (0.3)	0	-	-	-
e3	c.538C>T (p.R180C)	0	1 (0.1)	-	-	-
e3	c.542G>A (p.R181H)	1 (0.3)	0	-	-	-
i1	IVS1-5T>A	1 (0.3)	0	-	-	-
i2	IVS2+24C>T	1 (0.3)	1 (0.1)	-	-	-
i2	IVS2-54C>T [b]	1 (0.3)	0	-	-	-
All alterations within coding sequence		14 (4.7)	18 (2.6)	1.82	0.89-3.71	0.12
Alterations excluding I157T		5 (1.7)	2 (0.3)	5.81	1.12-30.12	0.03
All alterations		17 (5.7)	19 (2.8)	2.11	1.08-4.13	0.04

[a] Common odds ratio (OR) estimate with 95% confidence interval (CI) and significance p by 2-sided Fisher's Exact Test; [b] This variant was erroneously referred to as IVS2-55C>T in our previous publications [14, 17].

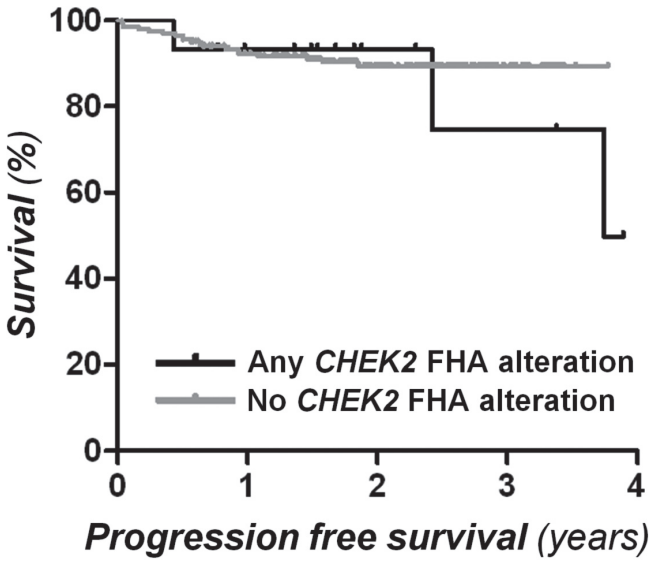

Figure. 1 Progression-free survival of HL patients in groups according to the presence of inherited *CHEK2* alterations. No significant difference in PFS was found (*p* values for Log-rank and Wilcoxon test were 0.53 and 0.97, respectively).

quency of *CHEK2* alterations in the group of HL patients (5.7%) differed significantly from that characterized previously [13] in controls (2.8%; $p = 0.04$). Presence of any alteration within analyzed region was associated with increased risk of HL development (OR = 2.11; 95% CI = 1.08 – 4.13). The most frequently occurring *CHEK2* alteration – c.470T>C (p.I157T) – was found in 4.0% of HL patients and 2.5% of controls (p=0.22). The frequency of other alterations (excluding the most frequent I157T mutation) was significantly higher in HL cases and associated with increased risk of HL development (OR = 5.81; 95% CI = 1.12 – 30.12; Table 2). Progression-free survival in HL patients did not differ between *CHEK2* alteration carriers and patients without alteration in analyzed region (Figure 1). Moreover, *CHEK2* FHA alterations did not correlate with any of clinical characteristic mentioned in Table 1.

Except for the c.542G>A (p.R181H) mutation, all other identified alterations were previously found in Czech breast, colorectal or pancreatic cancer patients [13, 14, 17]. The R181H was identified in breast and prostate cancer patients from Germany [18] and the USA [19], respectively, however, this variant most likely do not interfere with the function of the CHK2 (Align GVGD: Class C0) and together with c.538C>T (p.R180C – identified in one control subject) may represent neutral *CHEK2* sequence variants. Alteration c.475T>C (p.Y159H – previously described in one Czech breast cancer patient) affects highly conservative amino acid residue within the FHA-coding region (Align GVGD: Class C65) potentially influencing protein function [13]. Based on the computer prediction made in our previous studies, we deduced that intronic variants IVS1-5T>A and IVS2+24C>T may interfere with binding sites of splicing factors [13] and that IVS2-54C>T alters the most probable branching site [14], which both could lead to the aberrant splicing of *CHEK2* mRNA, however, these hypotheses have not been confirmed using functional *in vitro* analyses so far. The most frequent c.470T>C (p.I157T) variant is localized within the conserved sequence of *CHEK2* FHA domain. Despite the fact that based on Align GVGD this mutation belongs to the group of variants with limited impact (Class C25), the functional analyses clearly shown that mutated I157T CHK2 protein is defective in ability to bind some of its protein targets including p53 [20] or BRCA1 [21] proteins *in vitro* and due to the retained dimerization capacity the I157T heterozygotes exerts impaired substrate binding *in vivo* [22]. Alongside other previously mentioned solid cancers, the I157T mutation has been also associated with several lymphoproliferative diseases. Rudd et al. [23] described higher risk of chronic lymphocytic leukemia development (OR = 14.83; $p = 0.0008$) in carriers of I157T and Cybulski et al. [12] found increased frequency of I157T in non-Hodgkin lymphoma patients (OR = 2.0; $p = 0.05$).

This is the first study evaluating the potential impact of *CHEK2* I157T and other alterations localized within FHA domain or in its proximity on the risk of HL development. Though we are aware of limited power of our study due to the small number of tested patients, we showed that mutations in this *CHEK2* region represent mild but significant genetic factor increasing the risk of HL in mutation carriers. These results extend our previous observations from studies in breast and colorectal cancer patients that *CHEK2* FHA domain-coding region is affected by numerous distinct rarely-occurring alterations that together with predominant I157T mutation contribute to increased risk of many solid tumors and also at least several lymphoproliferative malignancies, including HL. However, further evaluation of *CHEK2* alterations in HL patients by larger studies is needed.

Acknowledgement. This work was supported by research grant of Grant Agency of Charles University in Prague No. GAUK 33508 and Research Project of the Ministry of Education, Youth and Sports of Czech Republic No. MSM0021620808 and No. MSM0021620813. We thank to Dr. Drahomira Springer providing the control blood samples, and hematologists and their patients for their collaboration. The special thank belongs to Ing. Jaroslava Hajkova for great help with isolation of DNA samples, to Mrs Helena Achylisova for sample handling and to Ing. Stanislav Kormunda for help with statistical analyses.

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