IJC
International Journal of Cancer

CHEK2 mutations and the risk of papillary thyroid cancer

Monika Siołek[1], Cezary Cybulski[2], Danuta Gąsior-Perczak[3], Artur Kowalik[4], Beata Kozak-Klonowska[1], Aldona Kowalska[3], Małgorzata Chłopek[4], Wojciech Kluźniak[2], Dominika Wokołorczyk[2], Iwona Pałyga[3], Agnieszka Walczyk[3], Katarzyna Lizis-Kolus[3], Ping Sun[5], Jan Lubiński[2], Steven A. Narod[5] and Stanisław Góźdź[1,6]

[1] Department of Genetics, Holycross Cancer Centre, Kielce, Poland
[2] Department of Genetics and Pathology, International Hereditary Cancer Centre, Pomeranian Medical University, Szczecin, Poland
[3] Department of Endocrinology and Nuclear Medicine, Holycross Cancer Centre, Kielce, Poland
[4] Department of Molecular Diagnostics, Holycross Cancer Centre, Kielce, Poland
[5] Centre for Research on Women's Health, Toronto Ontario, Canada
[6] Faculty of Health Sciences, The Jan Kochanowski University, Kielce, Poland

Mutations in the cell cycle checkpoint kinase 2 (*CHEK2*) tumor suppressor gene are associated with multi-organ cancer susceptibility including cancers of the breast and prostate. A genetic association between thyroid and breast cancer has been suggested, however little is known about the determinants of this association. To characterize the association of *CHEK2* mutations with thyroid cancer, we genotyped 468 unselected patients with papillary thyroid cancer and 468 (matched) cancer-free controls for four founder mutations of *CHEK2* (*1100delC, IVS2 + 1G>A, del5395* and *I157T*). We compared the family histories reported by patients with a *CHEK2* mutation to those of non-carriers. A *CHEK2* mutation was seen in 73 of 468 (15.6%) unselected patients with papillary thyroid cancer, compared to 28 of 460 (6.0%) age- and sex-matched controls (OR 3.3; $p < 0.0001$). A truncating mutation (*IVS2 + 1G>A, 1100delC or del5395*) was associated with a higher risk of thyroid cancer (OR = 5.7; $p = 0.006$), than was the missense mutation *I157T* (OR = 2.8; $p = 0.0001$). *CHEK2* mutation carriers reported a family history of breast cancer 2.2 times more commonly than non-carriers (16.4% *vs*.8.1%; $p = 0.05$). A *CHEK2* mutation was found in seven of 11 women (63%) with multiple primary cancers of the breast and thyroid (OR = 10; $p = 0.0004$). These results suggest that *CHEK2* mutations predispose to thyroid cancer, familial aggregations of breast and thyroid cancer and to double primary cancers of the breast and thyroid.

Cancer Genetics

Thyroid cancer is one of the most common cancers in young women.[1] The most common type of thyroid cancer is papillary thyroid cancer, which comprises 80% of all thyroid tumors.[2] Approximately 5% of cases of papillary thyroid cancer are familial.[3] Epidemiologic data suggests that inherited factors contribute to papillary thyroid cancer.[4] A familial association between thyroid and breast cancer has been suggested; a prior history of thyroid cancer is a risk factor for breast cancer and *vice versa* (relative risks in the range of 1.7–12.4).[5–11] However, outside of a rare inherited syndrome of cancer susceptibility called Cowden syndrome (caused by *PTEN* mutations) in which both breast and thyroid cancer risks are increased, the genetic basis for the association between these two cancers has not been established.[12]

Key words: CHEK2, CHK2, mutation, thyroid cancer, breast cancer, double primary cancers
DOI: 10.1002/ijc.29426
History: Received 7 July 2014; Accepted 17 Dec 2014; Online 12 Jan 2015
Correspondence to: Cezary Cybulski, International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, ul. Połabska 4, 70-115 Szczecin, Poland, Tel.: +[00-48-91-466-1532], Fax: +[00-48-91-466-1533], E-mail: cezarycy@sci.pam.szczecin.pl

Cell cycle checkpoint kinase 2 (CHEK2) is a key component of the DNA damage pathway.[13–16] Four founder alleles of *CHEK2* are present in Poland, three of these result in a truncated CHEK2 protein (*c.1100delC, c.444 + 1G>A, del5395)* and the fourth (*c.470T>C*) is a missense substitution of an isoleucine for a threonine in exon 3.[17] All these alleles were associated with multi-organ cancer susceptibility, including cancers of the breast and thyroid.[18–20] In the current study, we wished to better characterize the association between *CHEK2* mutations and thyroid cancer. We genotyped 468 unselected patients with papillary thyroid cancer and 468 matched cancer-free controls for four founder mutations of *CHEK2*. We also explored whether *CHEK2* mutations contribute to familial aggregation of breast and thyroid cancers and to cases with double primary cancers of the breast and thyroid.

Material and Methods

Patients

We studied 468 unselected patients with papillary thyroid cancer (44 men and 424 women; age at onset from 14 to 76 years, mean 48.2 years) diagnosed in a single oncology center, Kielce, Poland between 1999 and 2013. Patients were enrolled between 2010 and 2014 during their control visits in oncology outpatient clinics. Of the 1,554 cases diagnosed between 1999 and 2013 in Kielce, 468 patients (30.1%) had

What's new?
Cell cycle checkpoint kinase 2 (CHEK2) is an important component of the DNA damage pathway and a protein frequently mutated in cancer. Here the authors investigated the genetic association between thyroid cancer and *CHEK2* founder mutations in Poland. They find evidence that *CHEK2* mutations, especially truncations, predispose to papillary thyroid cancer. Interestingly, carriers of *CHEK2* mutations had frequent family histories of breast cancer and more than half of women with primary breast and thyroid cancers had *CHEK2* mutations, pointing to a possible causative role of these mutations in the aggregation of these cancers.

control visits between 2011 and 2014, and were included in the current study. All patients provided signed informed consent and a blood sample for DNA analysis. The time from diagnosis to blood draw was from 1 month to 14 years. Cases were unselected for age and family history.

The diagnosis of papillary thyroid cancer was confirmed through histopathological examination. Clinicopathological information was obtained from the medical records, and tumor, lymph nodes, metastasis (TNM) staging was recorded. A family history was taken by the construction of a family tree for all patients. All cancer cases in first- and second-degree relatives, and their ages of onset, were recorded. The information on age at diagnosis and family history were recorded from all cases at study entry, when blood sample was drawn. A detailed family tree was drawn by a physician. Three hundred thirteen of 468 cases (67%) reported a positive family history of (any) cancer.

A personal history of cancer at other sites was also collected. Thrity-two of the 468 patients (6.8%) with thyroid cancer had a second primary cancer (prior to or after the thyroid cancer). Twenty-nine women had a primary cancer other than thyroid: breast cancer (11 women), endometrial cancer (four women), skin cancer (three women), cervical cancer (two women), lung cancer (two women), other (seven women). Three men with papillary thyroid cancer had a second tumor (prostate cancer, testicular tumor, pheochromocytoma).

We studied 468 controls (age range 14–76; mean 48 years). Controls were cancer-free adults seen at the same hospital as the cases, and were matched 1:1 to the cases by sex and age (within 3 years). Controls were selected from two sources. Four hundred twenty-four female controls were selected from a group of women who participated in a mammography screening program in Kielce between 2007 and 2012 and who provided blood sample for DNA analysis between 2007 and 2012 and 44 male controls were selected from a group of men who were counseled in the Department of Genetics in Kielce between 2007 and 2013. The frequency of a *CHEK2* mutation (four mutations combined) in the 468 controls (6.0%) was similar to that reported previously in a large series of 8,302 adult controls from Poland (5.8%)[21]. All patients and control subjects were white.

Genotyping

Mutation analysis for the common mutations in the Polish population was performed as described previously.[17,22] In brief, the *CHEK2 del5395* mutation was detected by a multiplex polymerase chain reaction (PCR). The *IVS2 + 1G>A* and *I157T* variants were detected by restriction fragment length polymorphism (RFLP) PCR analysis, and the *1100delC* mutation was analyzed using an allele specific oligonucleotide (ASO) PCR assay. For samples in which a mutation was detected, DNA sample was sequenced to confirm the presence of the mutation. A positive and negative control was used for each analysis.

Statistical analysis

The frequencies of the four *CHEK2* alleles in cases and controls were compared. The four *CHEK2* variants were considered separately, and in combination. Odds ratios were used as estimates of relative risk of *CHEK2* variants and other related factors. To assess statistical significance, the Fisher exact test or the χ^2 test or a conditional logistic regression was used where appropriate. All reported *p*-values were two-sided. For all statistical analyses, a p values < 0.05 was considered significant. Subgroups of interest included by age of onset, by family history, by histologic subtype and by age. SAS statistical software was used for the analyses (version 9.3, SAS Institute Inc., Cary, NC).

Results

A *CHEK2* mutation (any of four mutations) was seen in 73 of 468 (15.6%) unselected patients with papillary thyroid cancer, compared to 28 of 468 (6.0%) age- and sex-matched controls (OR 3.3; 95% CI 2.0–5.4; $p < 0.0001$) (Table 1). A *CHEK2* mutation was present in 13.6% in 44 men with thyroid cancer and in 15.8% in 424 women with thyroid cancer. A *CHEK2* mutation was seen in 14.3% of 244 patients diagnosed at age 50 or below, and in 16.9% of 224 patients with thyroid cancer diagnosed at age above 50.

Both truncating and missense mutations were associated with increased risks of thyroid cancer. A truncating mutation (*IVS2 + 1G>A, 1100delC* or *del5395*) was seen in 17 of 468 (3.6%) unselected cases, compared to 3 of 468 (0.6%) controls (OR = 5.7, 95%CI = 1.7–19.3; $p = 0.006$). The missense mutation *I157T* was detected in 60 cases (12.8%), compared to 25 (5.3%) controls (OR = 2.8; 95% CI 1.7–4.6; $p = 0.0001$). A test for homogeneity of the two odds ratios was not significant ($p = 0.2$). *CHEK2* mutation carriers ($n = 73$) and non-carriers ($n = 395$) were similar with respect to age of

Table 1. Association of variant alleles in *CHEK2* with thyroid cancer risk

	Unselected cases ($n = 468$) no. (%)	Controls ($n = 468$) no. (%)	OR	95% CI	*p*-value
Any *CHEK2* mutation	73 (15.6%)	28 (6.0%)	3.3	2.0–5.4	<0.0001
Any *CHEK2* truncating mutation	17 (3.6%)	3 (0.6%)	5.7	1.7–19.3	0.006
1100delC	1 (0.2%)	0 (0.0%)	–	–	1.0
IVS2 + 1G>A	10 (2.1%)	1 (0.2%)	10.0	1.3–78.1	0.03
del5395	6 (1.3%)	2 (0.4%)	3.0	0.6–14.8	0.2
CHEK2 I157T missense mutation	60 (12.8%)	25 (5.3%)	2.8	1.7–4.6	0.0001

CI = confidence interval; OR = odds ratio.
Odds ratios, 95% confidence intervals and *p*-values were estimated by conditional logistic regression on the 468 matched pairs.

Table 2. Clinical characteristics of thyroid cancers in *CHEK2* carriers *vs* non-carriers

Factor	*CHEK2* mutation positive cases ($n = 73$)	*CHEK2* mutation negative cases ($n = 395$)	*p*-value
Age of diagnosis			
Mean (range)	49.5 (17–71)	47.9 (14–76)	0.3
≤40	16.4% (12/73)	27.1% (107/395)	0.06
41–50	31.5% (23/73)	25.8% (102/395)	0.3
51–60	39.7% (29/73)	31.9% (126/395)	0.2
>60	12.3% (9/73)	15.2% (60/395)	0.6
Staging			
pT1	61.1% (41/64)	64.2% (231/360)	1.0
pT2	10.9% (7/64)	9.2% (33/360)	0.6
pT3	18.8% (12/64)	21.4% (77/360)	0.7
pT4	6.2% (4/64)	5.3% (19/360)	0.8
Unknown	12.3% (9/73)	8.9% (35/395)	0.4
Lymph node status			
N1	20.0% (4/20)	22.1% (38/172)	1.0
No	80.0% (16/20)	77.9% (134/172)	1.0
Nx	72.6% (53/73)	54.4% (223/395)	0.01
Distant metastases			
M1	0.0% (0/5)	8.8% (3/34)	1.0
Mo	100% (5/5)	91.2% (31/34)	1.0
Mx	93.1% (68/73)	91.4% (361/395)	0.8

p-Values for mutation carriers are calculated with respect to non-carriers as reference group.

diagnosis, tumor stage lymph node status and distant metastases (Table 2).

We compared cancer family histories (in first or second degree relatives) reported by the carriers of a *CHEK2* mutation to those reported by the non-carrier cases. A positive family history of any type of cancer was more common in first degree relatives of carriers than in non-carriers (54.8% *vs*.39.2%; $p = 0.01$) and in second degree relatives (54.8% *vs* 44.6%; $p = 0.1$). Looking at specific cancer types, carriers reported more frequently a family history of breast cancer (16.4% *vs* 8.1%; $p = 0.05$), lymphoma (5.5% *vs* 1.0%; $p = 0.02$) and sarcoma 8.4 (4.1% *vs* 0.5%; $p = 0.03$) compared to non-carriers. Carriers *vs*. non-carriers also reported more frequently relatives affected with thyroid cancer (6.8% *vs*. 3.5%; $p = 0.2$), prostate cancer (11% *vs*. 5.8%; $p = 0.1$), and endometrial cancer (2.7% *vs*. 0.8%; $p = 0.2$), but for none of these was the difference statistically significant.

Of the 73 patients with thyroid cancer and a *CHEK2* mutation, 12 (16.4%) had (prior or past) diagnosis of another primary cancer (breast cancer—7 women, endometrial cancer—2 women, ovarian cancer—1 woman, skin cancer—1 woman, lung cancer—1 woman), compared to 20 of 395 (5.1%) non-carriers ($p = 0.001$) (Table 3). Of 15 different primary cancers seen in patients with papillary thyroid cancer, only breast cancer was seen in significant excess in *CHEK2* mutation carriers, compared to non-carriers—seven of 67 women with a *CHEK2* mutation (10.4%) was diagnosed with both breast and thyroid cancer versus only four of 357 (1.1%) women who did not carry a *CHEK2* mutation (OR = 10.3; $p = 0.0004$).

Seven of 11 (63%) women with double primary cancers of the breast and thyroid had a mutation (compared to only 6% of 460 healthy controls; OR = 10; $p = 0.0004$).

Six of 468 (1.3%) patients carried two *CHEK2* mutations (four women carried both *IVS2 + 1G>A* and *I157T*, and two women carried homozygous *I157T* mutation) compared to none of 460 controls ($p = 0.03$). Characteristic of thyroid cancers in carriers of two *CHEK2* mutations was similar to that seen in carriers of single mutations. Carriers of double mutations were diagnosed with thyroid cancer at mean age of 49.8 years (range 19–68 years). Two (33%) reported other primary cancer (lung and skin cancer). Five (83%) were diagnosed with Stage I tumors and one (17%) was diagnosed with Stage III tumor. Four (67%) reported 1st or 2nd degree relative with cancer.

Discussion

The *CHEK2* gene encodes the human analogue of the yeast checkpoint kinases Cds1 and Rad53.[15] Activation of *CHEK2* in response to DNA damage prevents the cell from entering into mitosis. Germline mutations in *CHEK2* have been associated with a moderate or low increase in the risk of a range of cancers.[20–28] Truncating mutations confer higher risks of

Table 3. Frequency of double primary cancers seen in *CHEK2* mutation carriers *vs* non-carriers

Other primary malignancy	*CHEK2* mutation positive patients, $N = 73$[1]	*CHEK2* mutation negative patients, $N = 395$[2]	OR	95% CI	*p*-value
Yes (any cancer type)	12/73 (16.4%)	20/395 (5.1%)	3.7	1.7–7.9	0.001
Breast cancer	7/67 (10.4%)	4/357 (1.1%)	10.3	2.9–36.2	0.0004
Endometrial cancer	2/67 (3.0%)	1/357 (0.28%)	11.9	0.97–122	0.07
Ovarian cancer	1/67 (1.5%)	0/357 (0.0%)	16.9	0.7–420	0.1
Skin cancer	1/73 (1.4%)	2/395 (0.5%)	2.7	0.2–30.5	0.4
Lung cancer	1/73 (1.4%)	1/395 (0.25%)	5.4	0.3–88.5	0.3

[1]Includes 67 women and 6 men with thyroid and a CHEK2 mutation.
[2]Includes 375 women and 38 men with thyroid cancer and without a CHEK2 mutation.

breast, prostate cancer and stomach cancer (2–3 fold) than does the missense mutation *I157T* (1.5-fold), but for colon cancer and kidney cancer, only the missense allele appears to be pathogenic.[20,28]

In 2004, we studied 173 thyroid cancers patients from a single center in Szczecin.[20] Here we extend our initial findings in a new non-overlapping series of 468 patients with thyroid cancer and 460 age- and sex-matched controls from Poland. In the current series, a *CHEK2* truncating mutation (*1100delC, IVS2 + 1G>A, del5395*) was associated with odds ratio of 5.7 ($p = 0.006$), and a *CHEK2* missense *I157T* mutation was associated with odds ratio of 2.8 ($p = 0.0001$) for thyroid cancer. These odds ratios are similar to those reported in our earlier study. In aggregate, these data provide evidence that both truncating and missense mutations of *CHEK2* confer thyroid cancer risk, and that a truncating mutation appears to confer a higher risk than the missense mutation. Of note, the association between the *I157T* allele and thyroid cancer was also confirmed in a recent study from central Poland of 1,781 patients with papillary thyroid cancer and 2,081 healthy control reported odds ratio of 2.2 ($p < 0.0001$), but truncating mutations were not analyzed and data on family history and other primary cancers were not provided.[29]

CHEK2 mutations are associated with a number of cancers; we found that a positive family history of breast cancer, lymphoma and sarcoma was more common in *CHEK2* carriers than in non-carriers and the difference was significant ($p < 0.05$). Also carriers reported more frequently family history of thyroid cancer, prostate cancer and endometrial cancer. These results further support that *CHEK2* is a cancer susceptibility gene associated with increased risk of cancer at several different sites, including breast, prostate, thyroid, colon, kidney, stomach, (low-grade) ovarian, bladder, chronic lymphocytic leukemia, Hodgkin lymphoma and a reduced risk of other cancers (lung and laryngeal cancer).[30–36] The current study suggests that the risk of sarcoma and non-Hodgkin lymphoma may also be increased. Of note, *CHEK2* mutations were first detected in patients with Li-Fraumeni type families, and in the original studies of *CHEK2* mutations and Li-Fraumeni syndrome, cancers of the breast, colon, ovary, endometrium, kidney, stomach were reported.[37] There is increasing evidence that *CHEK2* alone does not cause Li-Fraumeni syndrome, however, it might be a factor contributing (in combination with its modifying gene(s)) to individual tumor development in *TP53*-negative cancer-prone families.[38,39]

A genetic association between breast and thyroid cancer has been suggested, however outside *PTEN* mutations little is known about genetic determinants of this association. A previous study screened coding sequence of *PTEN*, *BRCA1* and *BRCA2* genes in 53 women with both breast and thyroid cancer, and only one *BRCA1* mutation was detected in the 53 women (2%) and no mutation was seen in *PTEN* and *BRCA2*.[40] In contrast, in our study, we saw a *CHEK2* mutation in 63% of 11 women with thyroid and breast cancer (compared to only 6% of 460 healthy controls; OR = 10; $p = 0.0004$). In addition, *CHEK2* carriers reported family history of breast cancer more commonly than non-carriers (16.4% *vs* 8.1%; OR =2.2; $p = 0.05$). This suggests that aggregation of breast and thyroid cancer may be commonly caused by *CHEK2* mutations and this association should be sought in other populations. The distribution of *CHEK2* mutations (in particular *1100delC* and *I157T*) is world-wide[20,32] and these and other *CHEK2* variants may be important contributor to thyroid and breast cancer in Poland and elsewhere.

The major truncating founder mutation of *CHEK2* gene is *1100delC*, which is seen with the frequency of 0.2%–1.4% in European populations,[18–25] but the allele appears to be infrequent in North America.[23] In our current study, *CHEK2 1100delC* mutation was present in only 1 of 468 patients with thyroid cancer, and in none of the 460 controls. In our previous study, this mutation was seen in 1 of 173 patients with thyroid cancer and in 10 of 4,000 controls from Poland.[20] When the data from these two studies are combined, the *1100delC* mutation was seen in 2 of 641 (0.3%) cases of thyroid cancer and in 10 of 4,460 (0.2%) controls (OR = 1.4, 95% CI 0.3–6.4, $p = 0.7$). Therefore, the risk for papillary thyroid cancer conferred by this particular mutation is uncertain and further studies are needed in this regard.

Acknowledgement

Authors thank Daria Zanoza for her help in this study. All authors declare no conflict of interest.

References

1. Preston-Martin S, Franceschi S, Ron E. Thyroid cancer pooled analysis from 14 case–control studies: what have we learned? *Cancer Causes Control* 2003;14:787–9.
2. Dong C, Hemminki K. Modification of cancer risks in offspring by sibling and parental cancers from 2,112,616 nuclear families. *Int J Cancer* 2001;92:144–50.
3. Pal T, Vogl FD, Chappuis PO, et al. Increased risk for nonmedullary thyroid cancer in the first degree relatives of prevalent cases of nonmedullary thyroid cancer: a hospital-based study. *J Clin Endocrinol Metab* 2001;86:5307–12.
4. Manolio TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. *Nature* 2009;461:747–53.
5. Adjadj E, Rubino C, Shamsaldim A, et al. The risk of multiple primary breast and thyroid carcinomas. *Cancer* 2003;98:1309–17.
6. Chen AY, Levy L, Goepfert H, et al. The development of breast carcinoma in women with thyroid carcinoma. *Cancer* 2001;92:225–31.
7. Li CI, Rossing MA, Voigt LF, et al. Multiple primary breast and thyroid cancers: role of age at diagnosis and cancer treatments (united states). *Cancer Causes Control* 2000;11:805–11.
8. Ronckers CM, McCarron P, Ron E. Thyroid cancer and multiple primary tumors in the SEER cancer registries. *Int J Cancer* 2005;117:281–8.
9. Rubino C, de Vathaire F, Dottorini ME, et al. Second primary malignancies in thyroid cancer patients. *Br J Cancer* 2003;89:1638–44.
10. Tucker MA, Boice JD, Jr, Hoffman DA. Second cancer following cutaneous melanoma and cancers of the brain, thyroid, connective tissue, bone, and eye in connecticut, 1935–82. *Natl Cancer Inst Monogr* 1985;68:161–89.
11. Vassilopoulou-Sellin R, Palmer L, Taylor S, et al. Incidence of breast carcinoma in women with thyroid carcinoma. *Cancer* 1999;85:696–705.
12. Ngeow J, Stanuch K, Mester JL, et al. Second malignant neoplasms in patients with cowden syndrome with underlying germline PTEN mutations. *J Clin Oncol* 2014;32:1818–24.
13. Chaturvedi P, Eng WK, Zhu Y, et al. Mammalian Chk2 is a downstream effector of the ATM-dependent DNA damage checkpoint pathway. *Oncogene* 1999;18:4047–54.
14. Matsuoka S, Rotman G, Ogawa A, et al. Ataxia telangiectasia–mutated phosphorylates Chk2 in vivo and in vitro. *Proc Natl Acad Sci USA* 2000; 97:10389–94.
15. Bartek J, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell* 2003; 3:421–9.
16. Ahn J, Urist M, Prives C. The Chk2 protein kinase. *DNA Repair (Amst)* 2004;3:1039–47.
17. Cybulski C, Wokołorczyk D, Huzarski T, et al. A large germline deletion in the Chek2 kinase gene is associated with an increased risk of prostate cancer. *J Med Genet* 2006;43:863–6.
18. Meijers-Heijboer H, van den Ouwel A, Klijn J, et al. CHEK2-breast cancer consortium. Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. *Nat Genet* 2002;31:55–9.
19. Meijers-Heijboer H, Wijnen J, Vasen H, et al. The CHEK2 1100delC mutation identifies families with a hereditary breast and colorectal cancer phenotype. *Am J Hum Genet* 2003;72:1308–14.
20. Cybulski C, Górski B, Huzarski T, et al. CHEK2 is a multiorgan cancer susceptibility gene. *Am J Hum Genet* 2004;75:1131–1135.
21. Teodorczyk U, Cybulski C, Wokołorczyk D, et al. The risk of gastric cancer in carriers of CHEK2 mutations. *Fam Cancer* 2013;12:473–8.
22. Cybulski C, Huzarski T, Górski B, et al. A novel founder CHEK2 mutation is associated with increased prostate cancer risk. *Cancer Res* 2004; 64:2677–2679.
23. Walsh T, Casadei S, Coats KH, et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. *JAMA* 2006;295:1379–1388.
24. Dong X, Wang L, Taniguchi K, et al. Mutations in CHEK2 associated with prostate cancer risk. *Am J Hum Genet* 2003;72:270–280.
25. Seppälä EH, Ikonen T, Mononen N, et al. CHEK2 variants associate with hereditary prostate cancer. *Br J Cancer* 2003;89:1966–1970.
26. Kleibl Z, Havranek O, Hlavata I, et al. The CHEK2 gene I157T mutation and other alterations in its proximity increase the risk of sporadic colorectal cancer in the czech population. *Eur J Cancer* 2009;45:618–24.
27. Kilpivaara O, Alhopuro P, Vahteristo P, et al. CHEK2 I157T associates with familial and sporadic colorectal cancer. *J Med Genet* 2006;43:e34
28. Cybulski C, Wokołorczyk D, Kładny J, et al. Germline CHEK2 mutations and colorectal cancer risk: different effects of a missense and truncating mutations? *Eur J Hum Genet* 2007;15: 237–41.
29. Wójcicka A, Czetwertyńska M, Świerniak M, et al. Variants in the ATM-CHEK2-BRCA1 axis determine genetic predisposition and clinical presentation of papillary thyroid carcinoma. *Genes Chromosomes Cancer* 2014;53: 516–23.
30. Brennan P, McKay J, Moore L, et al. Uncommon CHEK2 mis-sense variant and reduced risk of tobacco-related cancers: case–control study. *Hum Mol Genet* 2007;16:1794–801.
31. Cybulski C, Masojc B, Oszutowska D, et al. Constitutional CHEK2 mutations are associated with a decreased risk of lung and laryngeal cancers. *Carcinogenesis* 2008;29:762–5.
32. Wang Y, McKay JD, Rafnar T, et al. Rare variants of large effect in BRCA2 and CHEK2 affect risk of lung cancer. *Nat Genet* 2014;46:736–41.
33. Szymanska-Pasternak J, Szymanska A, Medrek K, et al. CHEK2 variants predispose to benign, borderline and low-grade invasive ovarian tumors. *Gynecol Oncol* 2006;102:429–31.
34. Złowocka E, Cybulski C, Górski B, et al. Germline mutations in the CHEK2 kinase gene are associated with an increased risk of bladder cancer. *Int J Cancer* 2008;122:583–6.
35. Rudd MF, Sellick GS, Webb EL, et al. Variants in the ATM-BRCA2-CHEK2 axis predispose to chronic lymphocytic leukemia. *Blood* 2006;108: 638–44.
36. Havranek O, Spacek M, Hubacek P, et al. Alterations of CHEK2 forkhead-associated domain increase the risk of Hodgkin lymphoma. *Neoplasma* 2011;58:392–5.
37. Bell DW, Varley JM, Szydlo TE, et al. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. *Science* 1999;286:2528–31.
38. Evans DG, Birch JM, Narod SA. Is CHEK2 a cause of the Li-Fraumeni syndrome? *J Med Genet* 2008;45:63–4.
39. Ruijs MW, Broeks A, Menko FH, et al. The contribution of CHEK2 to the TP53-negative Li-fraumeni phenotype. *Hered Cancer Clin Pract* 2009;7:4.
40. Pal T, Hamel N, Vesprini D, et al. Double primary cancers of the breast and thyroid in women: molecular analysis and genetic implications. *Fam Cancer* 2001;1:17–24.