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Short Communication

PENETRANCE OF *CHEK2* AND *BRCA1* DOUBLE HETEROZYGOTES IN BREAST AND/OR OVARIAN CANCER PATIENTS

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Germline pathogenic BRCA1 *variants confer increased risk of breast and/or ovarian cancer. The penetrance of* BRCA1 *pathogenic variants is variable due to the effects of other genetic factors. The interaction between CHEK2 and BRCA1 proteins is crucial in homology directed DNA repair pathway. The aim of this study was to assess the effect of three pathogenic/likely pathogenic variants of the* CHEK2 *gene on* BRCA1 *pathogenic allelic variant penetrance. The analysis included 380 DNA samples of women with confirmed positive* BRCA1 *status for one of founder variants c.4035del and c.5266dup. The c.444+1G>A and c.470T>C variants of* CHEK2 *gene were identified by Sanger's sequencing, and the del5395 variant was detected by multiplex PCR. The studied* CHEK2 *variants were found in 13 double heterozygous cases (c.444+1G>A, n = 1; c.470T>C, n = 11, del5395, n = 1). Although the prevalence of CHEK2 variants in the ovarian cancer group was comparatively high (5.41%), the increase of the ovarian cancer risk was not statistically significant (OR = 1.56; 95% CI: 0.32–9.94; p = 0.73). The association of the age at the onset of cancer with the presence of particular CHEK2 variant was not consistent.*

Keywords: BRCA1 *pathogenic variant carriers, breast cancer,* CHEK2*, double heterozygote, ovarian cancer.*

Breast cancer is the most common malignant tumour type among women. Although ovarian cancer is not so common, both remain a significant cause of death worldwide (Plakhins *et al.*, 2011; Sung *et al.*, 2021). Every year approximately 1200 and 300 women are diagnosed with breast and ovarian cancer in Latvia, respectively (SPKC, 2020). Heterozygous germline pathogenic variants in *BRCA1* gene and their associated hereditary breast and ovarian cancer (HBOC) is inherited in an autosomal dominant manner with incomplete penetrance, resulting in increased lifetime risk of breast and/or ovarian cancer. Homozygous or compound heterozygous germline pathogenic variants in the *BRCA1* gene are extremely rare and considered lethal during embryonic development. The cumulative risks of breast and ovarian cancer by the age of 70 for *BRCA1* pathogenic variant carriers have been estimated to lie in the range of 44% to 75% and of 43% to 76%, respectively (Walker *et al.*, 2017; Keupp *et al.*, 2019). The two most prevalent founder *BRCA1* variants c.4035del and c.5266dup constitute more than 80% of all identified pathogenic variants in Latvian breast and ovarian cancer patients (Loza *et al.*, 2021). The penetrance of *BRCA1* pathogenic variants is modified by various factors, that have to be taken into account during personalised risk assessment for prevention strategies, such as prophylactic surgery (Chen and Parmigiani, 2007).

The penetrance of *BRCA1* pathogenic variants is influenced by other genetic factors. *CHEK2* gene is a candidate gene for such effect as it is an upstream regulator of the *BRCA1* gene. Activation of CHEK2 and BRCA1 proteins as a part of DNA damage response prevents mitotic entry in the affected cells. In response to DNA double-strand breaks

ATM, protein phosphorylates CHEK2, thus activating it. The activated CHEK2 phosphorylates downstream effectors BRCA1 and TP53 proteins, increasing their tumour suppressor function (Meijers-Heijboer *et al.*, 2002; Cybulski *et al.*, 2004).

CHEK2 variants have been shown to predispose to the development of the cancer in some studies (Cybulski *et al.*, 2004; Walsh *et al.*, 2006). Four founder mutations in the *CHEK2* gene, i.e., three truncating variants (c.1100del, c.444+1G>A alias IVS2+1G>A, del5395) and one missense variant (c.470T>C (previously reported as I157T)), have been identified in the populations of Poland (Cybulski *et al.*, 2004; Cybulski *et al.*, 2006), the Czech Republic and Slovakia (Walsh *et al.*, 2006). Germline pathogenic *CHEK2* variants have been found in patients with various cancers, among them, breast, prostate and colorectal carcinoma patients (Cybulski *et al.*, 2004; Cybulski *et al.*, 2007; Gronwald *et al.*, 2009). However, the estimation of cancer risk in allelic variant carriers is still uncertain, as are the effects of various factors on the penetrance of *CHEK2* gene. Although these studies suggest that *CHEK2* variants are risk factors of low to moderate penetrance with higher frequency among cancer patients, previous studies found that the frequency of pathogenic *CHEK2* gene variants in colorectal, breast, ovarian, and prostate cancer patient populations was similar to those in the general population (Irmejs *et al.*, 2006; Plonis *et al*., 2015). The aim of this study was to determine whether the risk of breast and/or ovarian cancer is increased for *CHEK2* and *BRCA1* double heterozygotes.

In this retrospective study we analysed 380 DNA samples from the collection of RSU Institute of Oncology, which during the time period from August 2002 to June 2018 were isolated from peripheral blood of women with confirmed positive *BRCA1* status for only one of the two pathogenic frameshift variants (NM_007294.3:c.4035del (p.(Glu1346fs), rs80357711), localised on chromosome 17, *BRCA1* gene, exon 10) and NM_007294.3:c.5266dup (p.(Gln1756Profs), rs80357906), localised on chromosome 17, *BRCA1* gene, exon 19). Overall the study cohort consisted of 155 (40.79%) heterozygous carriers of the *BRCA1* pathogenic variant with diagnosed breast cancer (age 25–80), 126 (33.16%) heterozygous carriers of the *BRCA1* pathogenic variant with diagnosed ovarian cancer (age 28–79), and 99 (26.05%) heterozygous carriers of the *BRCA1* pathogenic variant with no approved diagnosis of any type of cancer at the time of the study and they constituted the control group for the study (age 22–71).

DNA was isolated from peripheral blood by the FlexiGene DNA Kit (Qiagen, Germany) in accordance with manufacturer's protocol. The pathogenic/likely pathogenic variants (Pavlovica *et al*., 2022) of *CHEK2* (splice site variant NM_007194.4:c.444+1G>A (p.(?), (rs121908698)), localised on chromosome 22, *CHEK2* gene, after exon 3) and missense variant NM_007194.4:c.470T>C (p.(Ile157Thr), rs17879961, localised on chromosome 22, *CHEK2* gene, exon 4)), were identified by Sanger's sequencing using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) with primers described elsewhere (Cybulski *et al.*, 2004). Sequencing results were analysed using a 3500 Genetic Analyzer (Applied Biosystems, USA). Data were analysed and edited with Sequencing Analysis Software and SeqScape™ Software (Applied Biosystems, USA) using Genome Reference Consortium Human Build 37 (GRCh37)/hg19 (released in 2009). The pathogenic NM_007194.4:c.(908+1_909-1)_(1095+1_1096-1)del *CHEK2* variant that results in exon 9-10 deletion (also reported as del5395) was detected by multiplex PCR (Veriti, Applied Biosystems, USA) as described earlier (Cybulski *et al.*, 2007; Plonis *et al.*, 2015); products of PCR reaction were separated in 2% agarose gel. The presence of deletion in multiplex PCR positive samples was confirmed by Sanger's sequencing. The method has been described in detail previously (Cybulski *et al.*, 2006).

For statistical analysis, the software program R, version 3.6.3, and Bioconductor package 'Survival', version 3.2-3, (Therneau, 2020) were used. Odds ratios and their statistical significance of variant prevalence in the study groups was assessed using the two-tailed Fisher's exact test. Additionally, to investigate the effect of the variants on the cumulative risk of breast and/or ovarian cancer, Kaplan-Meier estimates were used, and curve differences were tested by the Log-rank test. For cumulative hazard (time-to-event probability) prediction, Cox-regression analysis was performed.

The study cohort was formed by two subgroups on the basis of the particular founder allele of *BRCA1* gene. The overall study population consisted of 148 women carrying *BRCA1* c.4035del (41 in the breast cancer group, 66 in the ovarian cancer group and 41 in the control group) and 232 women carrying the *BRCA1* c.5266dup variant (114 in the breast cancer group, 60 in the ovarian cancer group and 58 in the control group). The penetrance of the *BRCA1* gene variants 4035del and 5266dup in the cohort was 28% for breast cancer and 45% for ovarian cancer, and 49% for breast cancer and 26% for ovarian cancer, respectively.

The studied *CHEK2* variants were discovered in 13 double heterozygous cases (c.444+1G>A, n = 1, c.470T>C, n = 11, del5395, n = 1), listed in Table 1. No sample contained two or more simultaneous *CHEK2* variants. To estimate the penetrance of *CHEK2* allelic variants for the development of breast or ovarian cancer in *BRCA1* pathogenic variant carriers, we compared the prevalence of the studied *CHEK2* variants in each cancer group and control group of the cohort. Although the prevalence of *CHEK2* variants was comparatively high (5.41%) in the ovarian cancer group, the increase of the ovarian cancer risk was not statistically significant (OR = 1.56; 95% CI: 0.32–9.94; $p = 0.73$). Also, the prevalence of the studied *CHEK2* variants among patients with breast cancer was not significantly higher in comparison to the control group (OR = 0.88; 95% CI: 0.15–6.15; $p = 1$).

The association of the age at the onset of cancer with the presence of a particular *CHEK2* variant was not consistent. For *BRCA1* c.4035del variant carriers, the median age at the

Table 1. Frequencies of *CHEK2* variants in the study cohort

Variant and case	No. of carriers/total	Frequency, %
c.444+1G>A		
Controls	1/87	1.15
BrCa Cases	0/132	0
OvCa Cases	0/111	0
c.470T>C		
Controls	2/87	2.30
BrCa Cases	3/132	2.27
OvCa Cases	6/111	5.41
del5395		
Controls	0/87	0
BrCa Cases	1/129	0.78
OvCa Cases	0/109	0

onset of any cancer was not affected by the *CHEK2* variant ($p > 0.3$ with Log-rank test). The median age at the onset of ovarian cancer for *BRCA1* c.5266dup carriers with any studied *CHEK2* variant was 8.5 years lower than for *BRCA1* c.5266dup carriers without the *CHEK2* variant. The hazard ratio for this effect was 3.93 (95% CI: 0.93–16.65). Although the observed difference reached statistical significance with the Log-rank test ($p = 0.043$) and we observed a trend for the association of confirmed *CHEK2* variants with younger age at the onset of ovarian cancer, the data did not show statistical significance using an alternative Cox regression model (regression coefficient: 1.37, $p = 0.064$).

An increasing number of studies suggests that the penetrance of pathogenic gene variants is frequently affected by allelic variants in other modifier genes. However, only a few studies have focused on patients with double heterozygous pathogenic variants of *CHEK2* and *BRCA1* genes in different populations. Only a small proportion of women are expected to be double heterozygotes, which was supported by the results of these studies, where thousands of patients, mostly with breast cancer, were analysed, and the *CHEK2* and *BRCA1* double heterozygotes were identified only in 1 to 15 cases, based on different *CHEK2* variant population frequencies (Meijers-Heijboer *et al.*, 2002; Cybulski *et al.*, 2009; Turnbull *et al*., 2012; Sokolenko *et al.*, 2014).

Previous studies have focused mainly on the comparison of *CHEK2* and *BRCA1* double heterozygote frequency among breast cancer patients in comparison to healthy controls. However, this study was designed to evaluate the assumption that *CHEK2* variants can affect *BRCA1* pathogenic variant penetrance. First, we evaluated *CHEK2* and *BRCA1* double heterozygotes in both breast, and ovarian cancer patients, and second, we compared them to the control group formed by the carriers of pathogenic *BRCA1* variant without diagnosis of any type of cancer at the time of the study.

In total, we observed *CHEK2* and *BRCA1* double heterozygotes in 13 cases, confirming the frequency of heterozygotes observed in previously mentioned studies. Although our results suggest a trend for association of a double heterozygous state of *CHEK2* and *BRCA1* variants with younger age at the onset of ovarian cancer, in comparison with a heterozygous state for *BRCA1* variant only, data did not demonstrate statistically significant effect of *CHEK2* variants on the penetrance of *BRCA1* pathogenic variants. These observations suggest that *CHEK2* pathogenic variants do not result in decreased age of cancer onset in *BRCA1* positive breast or ovarian cancer patients (Cybulski *et al.*, 2009; Sokolenko *et al.*, 2014, Sukumar *et al.*, 2021).

In this study, the observed frequency of the *CHEK2* del5395 variant (0.3%) in women with positive *BRCA1* status is lower than in *BRCA1* negative breast cancer patients (0.68%) and the control group (0.76%) of a previously reported study (Plonis *et al.*, 2015). Also, missense variant c.470T>C was observed less frequently in *BRCA1* positive breast cancer (2.27%) and control groups (2.30%) than reported in a previous study (Irmejs *et al.*, 2006), which reported 7.60% in breast cancer patients and 6.40% in healthy controls both without a diagnosed *BRCA1* variant. Similar trends were observed in the abovementioned studies, where a significantly lower frequency of *CHEK2* variants was observed in *BRCA1* positive breast cancer patients, while the *BRCA1* negative breast cancer cases had a higher frequency of *CHEK2* variants, suggesting negative interaction of these pathogenic variants (Meijers-Heijboer *et al.*, 2002; Cybulski *et al.*, 2009; Turnbull *et al*., 2012; Sokolenko *et al.*, 2014). This observation can be explained by the model where *CHEK2* and *BRCA1* double heterozygous cancer cells have reduced viability, in comparison to cells with only one pathogenic variant, as products of both genes are involved in the same DNA repair pathway. It has been suggested that the inhibition of CHEK2 protein can promote cell death in tumours with defects in the genes of DNA repair mechanisms, such as *BRCA1*, by increasing and accumulating DNA damage, and leading to cellular death. Also, the previous observation (Cybulski *et al.*, 2009) that *CHEK2* is overexpressed in *BRCA1* positive tumours supports the hypothesis that sufficient levels of wild-type CHEK2 are essential to maintain the viability of *BRCA1* positive cancer cells.

In this study we faced some limitations — a small number of patients with the double heterozygous genotype. Firstly, this can be explained with the limited cohort size (homogenous population of women with two most common *BRCA1* founder pathogenic variants in Latvia). Secondly, the frequency of *CHEK2* and *BRCA1* double heterozygotes is very rare. To verify the assumption that the double heterozygotes have higher breast or ovarian cancer risk requires a larger study cohort, for example some consortium sample analysis. The increased use of exome or whole genome sequencing in the future will allow to identify more cases of double heterozygotes, and meta-analysis will help to overcome the limitations of this study.

The evidence for the effect of pathogenic/likely pathogenic *CHEK2* variants on the risk of breast and/or ovarian cancer in the carriers of *BRCA1* pathogenic variants is inconclusive.

ETHICS

The study was approved by Rīga Stradiņš University and Pauls Stradiņš Clinical University Hospital boards, and the Central Medical Ethics Committee of Latvia. All individuals signed an informed consent form.

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CHEK2 UN *BRCA1* DUBULTHETEROZIGOTU PENETRANCE KRŪTS UN/VAI OLNĪCU VĒŽA PACIENTĒM

Krūts vēzis ir izplatītākais ar ļaundabīgiem audzējiem saistītais nāves cēlonis sieviešu populācijā Latvijā, un olnīcu vēzis ir nozīmīgs mirstības cēlonis. Viens no galvenajiem predispozīcijas faktoriem pārmantota krūts un/vai olnīcu vēža gadījumā ir *BRCA1* gēna patogēnais alēliskais variants. Bet tā kā šī slimība iedzimst ar nepilnīgu penetranci, tad ir svarīgi noteikt citu ģenētisko variāciju saistību ar krūts vai olnīcu vēža attīstības risku un to ietekmi uz slimības fenotipisku izpausmi. Šajā pētījumā tika analizēta trīs *CHEK2* gēna iespējami patogēnu/patogēnu variantu (c.444+1G>A, c.470T>C, del5395) ietekme uz *BRCA1* c.4035del un c.5266dup patogēno variantu penetranci 380 DNS paraugos. Pētījumā tika atklātas 13 *CHEK2* un *BRCA1* dubultheterozigotas pacientes (c.444+1G>A, n = 1; c.470T>C, n = 11, del5395, n = 1). Pētījuma pierādījumi par *CHEK2* variantu ietekmi uz krūts un/vai olnīcu vēža attīstības risku *BRCA1* pozitīvās pacientēs ir nepārliecinoši.