ASSOCIATION OF THE GENE CHEK2 MUTATIONS AND OTHER LOW-PENETRANCE GENES OF PREDISPOSITION TO THE DEVELOPMENT OF BREAST CANCER

S.O. Henyk-Berezovska¹, S.V. Klymenko²

¹SI «Institute of Hereditary Pathology of NAMSU», Lviv
²SI «National Research Center for Radiation Medicine of NAMSU”, Kyiv, Ukraine

Summary. The modern data on the association mutations of DNA repair CHEK2 and some other genetic polymorphisms with the risk of developing sporadic and hereditary/family breast cancer (BC) was analyzed. According to numerous studies in the presence of mutations CHEK2 (first of all mutations 1100delC) this risk increases in 2-3 times, and at presence of cancer family history – 4-5 times. Penetrance CHEK2 mutations can change (increase) other genetic disorders and the action of genotoxic environmental factors, including ionizing radiation. This, according to the authors, substantiates the feasibility study in Ukraine, peculiarities of CHEK2 polymorphism of persons exposed to ionizing radiation after the accident at the Chernobyl (cohort of women liquidators and evacuated from radiocontaminated territories, especially in those who develop breast cancer.

Key words: breast cancer, CHEK2 gene, mutation 1100delC, ionizing radiation,
One of the most serious consequences of human exposure to small doses of ionizing radiation is development of cancer diseases. According to the assessments of the UNSCEAR, approximately ten men exposed to radiation out of thousand have cancer of thyroid gland, and ten women out of thousand – breast cancer (BC) (per each Grey of individually absorbed dose) [1].

In the first 25 years after CNPP disaster, increased risk of development of leukemia was registered in liquidators of 1986-1987, and excess cases of BC were started to detect in women-“liquidators” [2]. Among all forms of solid tumors, the highest was increase of incidence of thyroid gland cancer – 5-6 times. However, according with the experience of monitoring of victims of Hiroshima and Nagasaki atomic bombings, it is possible after Chernobyl disaster that incidence of other cancer diseases – gastric and colorectal, lung cancer may grow in 20-40 years. Data on increase of mortality from solid malignant tumors among participants of liquidation of consequences of disaster and specific groups of population should be interpreted carefully and further control and scientific studies should improve our understanding of this effect [3].

Besides data of epidemiological studies, which confirm presence of causal link between development of BC and ionizing radiation, the idea is discussed that risk of development of radiation cancer increases with presence of genetic predisposition to its occurrence.

In particular, low-penetrance genes, polymorphism of which is significantly widespread in general population, can contribute to the development of radiation cancer [4]. To date, by frequency of prevalence (T, %) of allele, which causes certain cancer pathology, and by value of relative risk (RR) of the latter, genes are conventionally divided into the following groups: genes of high penetrance (RR > 8, T < 0.1 %) – BRCA1, BRCA2, TP53, PTEN; medium penetrance (RR > 3, T = 0.1 ± 10.0 %) – CHEK2, ATM, BRIP, PABL2; low penetrance (RR < 2, T > 20.0 %) – LSP1, CASP8, TNRC9, MAPK3K1, FGFR2, etc.

Of late years, several widespread low-penetrance genes, which cause susceptibility to malignant neoplasms, have been identified [5, 6]. Significant
achievement in study of BC pathogenesis was discovery of genetic polymorphisms (GP), which cause hereditary susceptibility to this disease. Since hereditary factors remain unknown in 75% of family BC cases, polygenic model was offered. It was presumed that in development of BC, many genetic loci may participate, each of them has poor effect, and combination of several of them causes the occurrence of hereditary susceptibility to disease [7]. For verification of hypothesis, series of scaled studies (with participation of many teams from countries of Western, Eastern Europe, USA and Asia) of BC association with different genomic variations – polymorphisms – have been planned and carried out (genome-wide association studies – GWAS). In these studies, hundred thousands of GP (single nucleotide replacements) have been studied and their association with BC in several thousands of patients has been determined [8]. It has been defined that polymorphisms rs2981582, rs1219648, rs1078806 of gene FGFR2; rs3803662, rs12443621 of gene TNRC9/TOX3, 16q12.1; rs889312 of gene MAP3K1, 5q11.2; rs3817198 of gene LSP1 are connected with risk of BC.

Polymorphism of DNA repair genes can impact the formation and stage of genetic instability of somatic cells after the effect of ionizing radiation. Determination of connection between risk of BC after exposure to ionizing radiation and polymorphic variants of gene CHEK2 as gene controlling integrity of DNA, as well as other low-penetrance genes, can elucidate peculiarities of development of radiation-associated cancer and contribute to the development of measures for its prevention.

Gene CHEK2, which is referred to genes of DNA repair, localizes on chromosome 22q12.1, codes protein CHEK2-protein kinase, which acts as tumor suppressor and is activated in response to the DNA damage, in particular, caused by ionizing radiation. CHEK2 plays key role in complex net of control of genome integrity “genome-surveillance”, which coordinates cellular cycle with DNA repair and survival or with cellular death. This surveillance realizes through functioning of series of proteins, which perform monitoring of cellular cycle in its certain checkpoints. In response to different forms of DNA damages caused by genotoxic
stresses, incomplete DNA repair can occur, and group of molecular cascades detects it. To DNA-damaging factors are referred environmental mutagens (chemical substances, ionizing radiation), as well as different endogenous reactive forms of oxygen that are formed during cellular metabolism. Mechanisms of monitoring and notification actualize fast “emergency service”, due to fast amplification of signals, which are transmitted from damaged DNA to monitoring effectors, which delay realization of cellular cycle and activate DNA repair.

Gene *CHEK2* contains approximately 50 kb of genomic DNA and consists of 14 exons. CHEK2-protein kinase is referred to the CDS1 group (serine-threonine kinases) and exists in the form of three isoforms. N-terminal domain under the impact of genotoxic factors phosphorylates with the help of ATM/ATR-kinases and performs regulatory function. FHA (Forkhead-associated) domain takes part in dynamic inter-protein interaction of CHEK2 at transmission of signals during the monitoring of cellular cycle. Kinase domain (SQ/TQ) occupies most of C-terminal CHEK2 part, contains the main functional element – activation node and is characterized by structural homology with different serine-threonine kinases.

CHEK2-protein kinase performs one of the key roles in the system “genome-surveillance”. Activation of CHEK2 occurs in the ATM-dependent mode via phosphorylation of threonine-68. Activated CHEK2 inhibits activity of phosphatase CDC25C, preventing entrance of cell in mitosis. As consequence, inhibiting phosphorylation and further degradation of members of CDC25 (A, B, C) family takes place that leads to inactivation of cyclin-dependent kinases (CDK2 in S phase or CDK1 in G2 phase) and stop of cellular cycle. CHEK2 phosphorylates tumor suppressor p53 that leads to the arrest of cellular cycle in the stage G1 or apoptosis. Moreover, CHEK2 phosphorylates BRCA1 launching DNA repair in response to the damage [9, 10, 11, 12, 13].

Researchers have identified several mutations in gene *CHEK2* – 1100delC, which prevents phosphorylation of protein: A347D and R145W, which are missense-mutations; R3W and I157T, functional consequences of which are not clearly determined [14].
Firstly, mutation of gene \textit{CHEK2} 1100delC was said to be connected with syndrome of Li-Fraumeni, which is characterized by extremely invasive phenotype of family cancer. In 1999, Bell et al. \cite{15} have discovered three embryonic \textit{CHEK2} mutations in four patients with classical Li-Fraumeni syndrome and in 18 families with similar signs to Li-Fraumeni syndrome with paradox lack of mutations in gene p53, having presumed that \textit{CHEK2} can be new susceptibility factor of this syndrome. However, next researches focused on study of p53-negative variants of Li-Fraumeni syndrome did not confirm these results, having determined that function of \textit{CHEK2} is necessary for support of chromosomal stability of somatic cells and is independent from p53 \cite{16, 17}.

Mutation \textit{CHEK2} 1100delC is most widespread and causes synthesis of defective reduced protein \textit{CHEK2} with decreased or lacking kinase activity. 1100delC-alleleic variant was determined in 5.1\% of BC patients from 718 West-European and North-American families without \textit{BRCA1} or \textit{BRCA2} mutations and in 1.1\% of healthy people. Authors have noted that presence of 1100delC-mutation causes double increase of BC risk in patients that constitutes approximately 1\% from all cases of this disease \cite{18}.

The highest population frequency of \textit{CHEK2} mutation – 1100delC was observed in Netherlands (1.3–1.6\%) and in Finland (1.1–1.4\%), the lowest – in Great Britain (0.35–0.5\%), Germany (0.15–0.25\%), Australia (0.14\%) \cite{19}; Sweden (0.7–1.0) \cite{20}, Poland (0.20–0.25\%) \cite{21, 22}, Czech Republic (0.27\%) \cite{23}, Italy (0.11\%), \cite{24}, USA (0.3–0.4\%) \cite{25, 26} and Canada (0.2\%) \cite{27}. In Spain, this mutation was not detected at all \cite{28}. In Basque country, mutation 1100delC was registered in 0.93\% case of BC and no cases – in control population \cite{29}. Studies of Chilean scientists \cite{30} did not detect mutation of gene \textit{CHEK2} 1100delC in families with family BC in population of South America. Modern population of Chile was formed through assimilation of South American Indians and Spanish colonizers in XVI-XVII centuries. Spanish colonizers came in Chile exactly from South of Spain, where this mutation was not detected. Since the highest frequency of \textit{CHEK2} mutation 1100delC was registered in North and West Europe, and the lowest – in the
South of Europe, there is a hypothesis of gradient-progressive presence of this mutation in direction from North Western to South Eastern Europe that is caused by its common hereditary origin in the North of Europe. It is connected with founder effect, and 1100delC is a mutation occurred in one of ancestor’s gametes (so called neomutation, or mutation *de novo*) and with lapse of time being passed on from generation to generation.

In Finland, mutation 1100delC was detected in 5.5% of 507 patients with family BC anamnesis (without *BRCA* mutations) compared with 1.4% of 1885 healthy individuals (control group) of Finnish population. Moreover, patients with bilateral BC 6 times more often were carriers of 1100delC allele, than patients with unilateral BC [31]. Screening for germinal mutations in 7 genes of susceptibility to development of BC *BRCA1, BRCA2, CHEK2, PALB2, BRIP1, RAD50, and CDH1* has been carried out in individuals with high risk of familial BC and (or) ovarian cancer with negative test on fundamental mutations *BRCA1* or *BRCA2* and in control group (384 healthy individuals from Finnish population). In 12.2%, presence of two allelic variants of gene *CHEK2* – 470T > C and (or) 1100delC were detected that indicates significant contribution of these mutations for individuals with high risk of BC, but segregation analysis is needed for evaluation of clinical significance of these mutations [32].

In 5 countries, genotyping of 1100delC allele in 10860 cases of BC and in 9065 control cases has been carried out (10 studies by “case-control” method). Allele 1100delC was detected in 1.9% of patients with BC and 0.7% of individuals of control group (odds ratio (OR) 2.34; 95% confidence interval (CI) 1.72–3.20). Higher frequency of 1100delC allele has been determined among women with diseased relatives of the first level of affinity (CI 1.44; 95% CI 0.93-2.23; p=0.10) and earlier development of disease (p=0.002) [19]. Earlier start of disease was observed in carriers of 1100delC mutation, rather than in patients without such mutation [33]. These results confirm hypothesis of multiplicative impact of *CHEK2* 1100delC allele and critical alleles of other genes on increase of BC risk.
According with the data of Chinese scientists [34], mutation 1100delC was not detected in 74 patients with family history of BC and in 50 healthy individuals, but in patients with family history of BC was detected missense-variant 1111C>T (His371Tyr) of gene CHEK2. Mutation 1100delC may be quite rare variant in Chinese population and probably does not cause susceptibility to familial BC in this country. In study of population of Siberian region of Russian Federation, higher frequency of 1100delC mutation has been determined – 1.78% in patients with BC compared with control group from Novosibirsk city – 0.40% (OR=4.46, 95% CI 2.04-9.49) [35].

Frequency of cancer incidence in first-degree relatives of 107 patients with CHEK2 1100delC-positive familial BC and first-degree relatives of 1314 patients with CHEK2 1100delC-negative familial BC in Netherlands has been compared. All patients were with negative test on mutation BRCA1/2. Medical information of 2188 first-degree relatives has been analyzed. Increased risk (IR) of BC was determined in sisters of patients with 1100delC-positive tumors compared with sisters of patients with CHEK2 1100delC-negative BC (IR= 2.0, 95% CI 1.4-2.7, P<0.001). For mothers of patients with CHEK2 1100delC positive cancer, risk was increasing 1.6 times (95% CI 1.0-2.4) compared with mothers of patients with 1100delC-negative BC (p= 0.041). Such results show increased risk of occurrence of cancer in first-degree relatives of patients with CHEK2 1100del-positive familial BC that points at necessity of genotyping of this mutation in families with BC in countries, where this mutation is widespread, for improvement of clinical results in such patients [36].

Range of cancer diseases associated with mutations CHEK2 can be wider, than previously was considered. Protein CHEK2 takes part in DNA repair by many types of cells and, therefore, can be considered gene of susceptibility to cancer in various organs. Point mutation I157T and deletion mutation 1100delC, which code reduced CHEK2-protein with decreased or lacking kinase activity is one of the main mutations, which increases risk of BC as well cancer of prostate gland, thyroid gland, urinary bladder, kidney, ovaries and intestine [12].
Hypothesis that CHEK2 is polyorganic gene of susceptibility to malignant transformation of cells is confirmed by data published by Poland scientists. Polyorganic increase of susceptibility to malignant tumors is associated with other genes involved in process of DNA repair including BRCA1 (MIM 113705), BRCA2 (MIM 600185) and NBS1 (MIM 602667). In Poland, frequency of 3 main alleles of gene CHEK2 – IVS2+1G>A, 1100delC, and I157T in 4008 cases of the most widespread cancer diseases and in 4000 control cases has been studied. These polymorphic variants are present in 5.5% of Polish residents. All three allelic variants are connected with increased risk of prostate cancer [22]. Statistically reliable increase of risk of thyroid gland cancer (OR 4.9; p = 0.0006), BC (OR 2.2; p = 0.02), prostate gland cancer (OR 2.2; p = 0.04), which is connected with two allelic variants of gene CHEK2 – main mutations – IVS2+1G>A and 1100delC, has been registered. Missense-mutation I157T was associated with increased risk of BC (OR 1.4; p = 0.02), intestine cancer (OR 2.0; p = 0.001), kidney cancer (OR 2.1; p = 0.0006), prostate gland cancer (OR 1.7; p = 0.002), and thyroid gland cancer (OR 1.9; p = 0.04).

Authors of the study forecast that effect of the main and missense-mutations are different. If prostate, breast and thyroid gland cancers are associated with mutations of both types, than kidney and colorectal cancers are associated with only CHEK2-missense variants, but not with main mutations of this gene [21].

In study carried out at the University of Szczecin (Poland, 2011), risk of BC in women with CHEK2-mutations with family cancer history and without it has been evaluated. Screening of 7494 BC patients with negative test on BRCA1-mutations and 4346 women of control group has been carried out for the 4 main mutations of gene CHEK2 (del5395, IVS2+1G>A, 1100delC and I157T). Shortened mutations (IVS2+1G>A, 1100delC, or del5395) were registered in 227 (3.0%) patients and 37 (0.8%) women from control group (OR 3.6; 95% CI 2.6 – 5.1). OR was higher in women, whose first-degree and second-degree relatives had BC (OR 5.0; 95% CI 3.3 – 7.6), than in women with lack of family history of disease (OR 3.3; 95% CI 2.3 – 4.7). Evaluating basic risk as 6.0%, author have determined lifetime risk of BC for
carriers of shortened CHEK2 mutations as 20.0% for women who have no diseased relatives; 28.0% for women with one diseased second-degree relative; 34.0% for women with one diseased first-degree relative and 44.0% for women with two diseased first-degree ad second-degree relatives. Thus, screening of mutations of gene CHEK2, which determines clinically significant risk of disease, should be carried out in all women with family history of BC. In women with shortened mutations CHEK2 and family history of cancer, lifetime risk of occurrence of disease is higher, than 25.0% [37].

Study carried out by the USA researchers from Michigan Cancer Center [38] has determined frequency of mutation CHEK2 1100delC in families with BC with negative test on mutation BRCA1/2. Genotyping of mutation 1100delC in 102 patients from 90 families with BC or ovarian cancer, as well as with other cancer pathology, has been carried out. No 1100delC mutation in each of the 102 patients, including 51 patient with BC determined in the young age (<45), 8 women with bilateral BC, 3 men with BC and 8 women with ovarian cancer, have been determined. These data are the evidence of very low frequency of mutation CHEK2 1100delC in population of North America compared with North Europe.

Carriers of 1100delC allele also have high risk of development of bilateral BC. Statistically reliable increase of risk of occurrence of secondary contralateral BC has been registered (OR = 6.5; 95% CI 1.5–28.8, p = 0.005). At that, higher percentage of carriers of this mutation was in group of patients with bilateral cancer, who received radiation therapy within the treatment of primary diagnosed BC. These results can point at clinical significance of interaction between heterozygosity of gene CHEK2 and ionizing radiation [39]. Radiation therapy can be risk factor of development of BC in carriers of 1100delC-allele of gene CHEK2. In the followed works of these authors [40], contribution of germinal mutations in genes of DNA repair BRCA1, BRCA2, CHEK2 and ATM to the risk of development of radiation-induced contralateral BC has been studied. Frequency of mutations was 24.3% among patients with contralateral BC, who were exposed to radiation therapy, and 12.8% among not exposed to radiation patients (OR 2.18; 95% CI 1.03 – 4.62, p = 0.043). Carriers of
germinal mutations in genes of DNA repair have increased risk of development of radiation-induced contralateral BC compared with non-carriers of these mutations (OR 2.51; 95% CI 1.03 – 6.10; p = 0.049) after performance of radiation therapy not less, than 5 years after the first diagnosis of BC. However, no higher frequency of 1100delC in German population among patients with family BC with family anamnesis of bilateral cancer was registered [41].

Large population examinations have been carried out in WECARE studies on epidemiologic aspects of cancer in women under the effect of environmental factors, in particular ionizing radiation [42]. Frequency of 1100delC mutation has been evaluated in 708 women with contralateral and 1395 women with unilateral BC. Seven (1.0%) women with contralateral and 10 (0.7%) women with unilateral BC were carriers of 1100delC-variant of gene CHEK2 (OR 1.8; 95% CI 0.6-5.4). No statistically significant correlation between status of carriers of 1100delC mutation and risk of contralateral cancer both in general cohort and among patients, who received radiotherapy, has been found. However, authors of studies pay attention on the fact that increase of risk of development of radiation-induced contralateral BC in carriers of these mutations, which are sensitive to DNA-damaging impact of radiotherapy, cannot be excluded. In other WECARE studies, increased risk of contralateral BC has been determined in carriers of RAD50 haplotype, who received dose more than $\geq1$ Gy (OR 2.13; 95% CI 0.61-5.33) [43].

Status of carrier of 1100delC-allele of gene CHEK2 can be associated with sensitivity to diagnostic impact of ionizing radiation (except mammography) according to the data of population studies of 2311 women with BC and 496 control individuals from general population carried out in Ontario and North California (USA) by data of Register of families with BC [27]. Mutation of 1100delC was detected in 30 (1.34%) women with BC and in 1 (0.20%) control case (OR 6.65; 95% CI 2.37-18.68). When stratifying examined individuals by demographic and anamnesis features, carriers of 1100delC mutation were found more often among women of Caucasian population, over the age of 45, who underwent diagnostic impact of ionizing radiation more than 15 years before the moment of BC diagnosis.
(OR 4.28; 95% CI 1.50–12.20), and among those who received two or more radiologic breast examinations (OR 3.63; 95% CI 1.25-10.52).

In study of our authors [44], assessment of risk of BC for daughters of women with bilateral BC depending on CHEK2 status in mothers has been carried out. Basing on standard genetic model, authors prognosticated lifetime risk of BC for daughters of carriers of 1100delC mutation on the level of 37.0%, for daughters of patients without this mutation – 18%. It means that clinical monitoring of first-degree relatives of women with bilateral BC should take into account status of carrier of mutation 1100delC of gene CHEK2 in their mothers.

In 2012-2013, studies GWAS have been started – full genome study of BC association with different genomic variations – genetic polymorphisms as factors of prognosis of development of this disease [45, 46]. By the results of meta-analysis of 46,747 BC cases and 87,342 control cases, in 16 studies by “case-control” method, statistically reliable increase of risk of cancer associated with allele rs2981582, rs1219648 and rs2420946 of gene FGFR2 has been detected [47]. Moreover, it has been determined that OR for these alleles was higher for patients with hormone-positive tumors, than with hormone-negative tumors. It correlates with data on involvement of gene FGFR2 in estrogen-dependent carcinogenesis of mammary gland and with higher level of expression of this gene in hormone-positive tumors [48]. Study of other authors has determined that genetic polymorphism rs3817198 of gene LSP1 is associated with increased risk of BC only for carriers of mutation of gene BRCA2, and presence of genetic polymorphism rs3803662 of gene TNRC9/TOX3 increases risk of development of cancer for carries of mutation BRCA1 [49, 50]. Twenty three studies carried out among more than 10,000 carriers of BRCA1 and BRCA2 mutations have determined presence of insignificant association with increased risk of BC for genetic polymorphism of rs889312 of gene MAP3K1 in carriers of mutation of gene BRCA2 and lack of connection between this polymorphism and risk of development of BC in carriers of BRCA1 mutation [51]. Thus, GWAS studies demonstrate new approach for identification of low-penetrance alleles, i.e. risk caused by these genetic polymorphisms is insignificant, but their
combination can cause occurrence of hereditary susceptibility to BC that will contribute to the development of new strategy of analysis of impact of single nucleotide genetic polymorphisms.

Thus, though genes CHEK2 (as well as LSP1, CASP8, TNRC9, MAPK3K1, FGFR2) are referred to medium-penetrance and low-penetrance, increase of risk of occurrence of BC caused, in particular, by mutations CHEK2 is quite high – 2-3 times, and in presence of family cancer history – 4-5 times [4, 5, 19]. Risks associated with genes LSP1, CASP8, TNRC9, MAPK3K1, TOX3, FGFR2 are quite low (1.1 – 1.3), but frequency of spread of these genes in heterozygote state is quite high (>20%). Moreover, their impact significantly increases in families with high risk of BC and ovarian cancer [8, 45, 46, 47].

In Ukraine, no studies have been carried out for determination of both population frequency of mutation of DNA repair gene CHEK2 and low-penetrance genetic polymorphisms and frequency of mentioned genetic features in individuals, who were exposed to the ionizing radiation after Chernobyl disaster and were taken with cancer. Data of foreign studies cited in this survey confirm hypothesis, according to which penetrance of CHEK2 mutations in families with high BC risk is modified by other genetic disorders and/or environmental factors (in particular, ionizing radiation). These data substantiate, in our opinion, expediency of determination of peculiarities of polymorphism of DNA repair gene CHEK2 and low-penetrance genetic polymorphisms in individuals, who underwent ionizing radiation after Chernobyl disaster (cohorts of women-liquidators of CNPP and evacuated from radiation-contaminated territories), especially in those taken with BC. Results of such studies will contribute to discovering of mechanisms of occurrence of cancer pathology under the effect of ionizing radiation, and also developing of measures for reducing its risk in the most sensitive to radiation individuals.

REFERENCES


