Low frequency of the *CHEK2*1100delC* mutation among breast cancer probands from three regions of Poland*

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The 1100delC germline mutation of the *CHEK2* gene appears to contribute significantly to the overall breast cancer incidence in some West and North European countries, but seems to be much less frequent among breast cancer patients from other regions of Europe. In the present study we found, respectively, 3/487, 1/296 and 0/279 carriers of this mutation among breast cancer patients from the East-Central, South-East and West-Central regions of Poland. Two carriers of the 1100delC mutation were found among 120 patients with bilateral breast cancer, but only one had a previous family incidence of breast cancer. We found no carriers among 182 patients with unilateral breast cancer with family history of this tumor and among 64 patients with breast cancer and a second primary tumor at an other site. We conclude that the 1100delC mutation of the *CHEK2* gene contributes little to the overall breast cancer burden in Poland, including familial cases of this malignancy. Further studies are still needed to evaluate the contribution of this mutation to the development of bilateral breast tumors.

*Key words: breast cancer, CHEK2*1100delC mutation, population differences*

The CHEK2 protein, a cell-cycle checkpoint kinase, which plays an important role inresulting DNA damage, contains three important domains: the N-terminal SQ/TQ domain (SCD) with multiple ATM recognition sites, a forkhead associated domain (FHA) required for protein-protein interactions and Ser/Thr kinase domain, which activates substrates. Following radiation-induced DNA damage, CHEK2 after phosphorylation by ATM, oligomerization and autophosphorylation phosphorylates several substrates, including Cdc25A, Cdc25C, p53, E2F-1, PML and BRCA1, thus leading to cell cycle arrest, apoptosis and DNA repair [1].

The *CHEK2* gene has 14 coding exons distributed over a 50-kb genomic region at chromosome 22q12. The last four exons of this gene have however multiple homologous copies in the human genome, which makes mutational analysis of these exons problematic [2].

A protein-truncating mutation 1100delC in exon 10, which abolishes the kinase function of CHEK2 [3], was first found in families with Li-Fraumeni syndrome (LFS) that do not carry *TP53* mutations [4]. Several subsequent studies have suggested a significant contribution of this mutation to familial breast cancer incidence. In particular, the 1100delC mutation was identified in 5.1–5.5% patients with familial *BRCA1/2* negative breast cancer versus 1.1–1.4% in controls from Northern Europe [5, 6]. Moreover, a still higher frequency of this mutation was found among patients with male breast cancer (13.5%) [7] and with bilateral breast cancer (12.1%) [6] from this region.

Recently, the CHEK2 Breast Cancer Consortium [8] found a 1.9% frequency of 1100delC mutation among 10 860 probands with breast cancer and in 0.7% of 9 065 healthy controls from five countries (UK, Netherlands, Finland, Germany, Australia). It was concluded in this study that carriers have only a twofold increased risk of breast cancer, although the frequency of *CHEK2*1100delC* was higher among probands with first-degree relatives with breast cancer. Other reports suggest much lower *CHEK2*1100delC* mutation frequencies in other regions of the world, as well as of Europe [9, 10, 11, 12, 13].

In a recent study from North-Western Poland, which is

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however mostly inhabited by immigrants from the former Eastern Poland now under Ukrainian and Byelorussian rule, CYBULSKI et al [7] found only 5 carriers of the 1100delC mutation among 1017 breast cancer probands.

We found in the present study that the above mutation is similarly infrequent among patients, both with sporadic and familial breast cancer, from three other major regions of Poland.

Material and methods

Patients. Altogether, we screened for the *CHEK2*1100delC* mutation 1062 breast cancer probands including 182 patients with familial breast cancer incidence (among them 49 patients with two or more first- or second degree relatives with breast cancer and 133 with one relative with this tumor), 120 patients with bilateral breast cancer (among them 54 patients with breast cancer in one or more relatives not included into other subgroups) synchronous (50) or metachronous (70) and 64 patients with a second primary tumor at other site (including 25 patients with both breast and ovarian cancer and 24 patients with breast and endometrial cancer).

Of these, 487 patients from East-Central Poland (Mazovian voivodship), 279 from West-Central Poland (Wielkopolska voivodship) and 296 patients from South-East of Poland (Malopolska voivodship) were either referred for treatment or for genetic counseling, respectively to the Maria Sklodowska-Curie Cancer Center in Warsaw, to the Wielkopolska Cancer Center in Poznan and to the Maria Sklodowska-Curie Cancer Center branch in Krakow. Except of patients with familial, bilateral or second primary tumors who were referred for genetic counseling, other probands were nonselected. All patients provided written informed consent for genetic testing.

Mutation analysis. Genomic DNA was extracted from peripheral blood or dried blood samples collected on Guthrie cards using "Genomic Midi AX" kit or "Genomic Mini AX Blood" kit (A&A Biotechnology, Gdansk, Poland) respectively, according to the manufacturers protocols. The 1100delC variant was analyzed by an allele-specific PCR assay, using primers and conditions as described by CYBULSKI et al [7], to avoid the amplification of homologous copies of the gene. Mutation positive and negative controls for the 1100delC mutation were run always with each set of samples screened. All positive samples and a part of negative samples were verified by dHPLC (WAVE, Transgenomic, Omaha, USA) or by direct sequencing on ABI Prism 377 Sequencer (Applied Biosystems, Foster City, USA) using templates independently amplified by a long-range PCR [14].

Results

As shown in Table 1, we found only 4 carriers of the *CHEK2*1100delC* mutation among the 1062 breast cancer patients screened. Only one of these carriers had a family history of breast cancer and belongs to the subgroup bilateral breast cancer. We found two carriers among 120 patients with bilateral breast cancer, significantly more then among patients with unilateral tumors. The difference between these two subgroups did not however attain statistical significance (p=0.0600 Fisher's Exact test). One of these carriers with metachronous breast cancer aged, respectively, 42 and 49 years at diagnosis of the first and second tumor, had a first degree relative with laryngeal cancer and three second-degree relatives with malignant tumors, respectively, of the breast at the age of 43, of the larynx at the age of 70 and of the lung at the age of 65 years. The second carrier with synchronous breast cancer at age 64 had no family history of malignant diseases. Median ages of all patients with bilateral breast cancer and sporadic breast cancer were respectively, 48 and 52.

Three carriers (two with bilateral and one with sporadic breast cancer) were found among 487 probands from East-Central Poland and one carrier (with sporadic breast cancer) was found among 296 patients from South-East of

Table 1. The frequency of the *CHEK2*1100delC* mutation among breast cancer probants from three regions of Poland

Study cohorts and subgroups	Number of carriers/number of probants			
	East-Central Poland (Mazowsze voivodship)	West-Central Poland (Wielkopolska voivodship)	South-East Poland (Malopolska voivodship)	Total
All breast cancer patients	3/487	0/279	1/296	4/1062
patients with relatives with breast cancer[a]	0/70	0/75	0/37	0/182
patients with bilateral breast cancer[b]	2/87	0/24	0/9	2/120
patients with a second primary tumor at other site[c]	0/9	0/52	0/3	0/64
patients with no family history of breast cancer[d]	1/321	0/128	1/247	2/696

[a]including 49 patients with two or more relatives with breast cancer and 133 patients with one relative with this tumor (probants with bilateral breast cancer with relatives with breast cancer were not included into this subgroup),
[b]including 54 patients with breast cancer in one or more relatives (50 tumors developed synchronously and 70 metachronously),
[c]including 25 patients with both breast and ovarian cancer, and 24 patients with both breast and endometrial cancer,
[d]including 43 patients with early onset of disease (≤35 y.).

Poland. We found no carriers among 279 patients from West-Central Poland. The ages of two carriers with sporadic unilateral breast cancer were, respectively, 52 and 47 years.

Discussion

The 1100delC mutation of the *CHEK2* gene contributes significantly to the overall incidence of sporadic and familial breast cancer in North and Western Europe [5, 6], but appears to be much less frequent in North America [10, 12], in Spain [13] and Italy [9]. Short of a study by CYBULSKI at al [7] from North-Western Poland and by KLEIBL et al [11] from the Czech Republic, little is known about the frequency of this mutation among breast cancer patients from the Slavonic populations of Central Europe.

Previous studies have demonstrated considerable interregional differences in Poland of the population frequencies of the 657del5 mutation of the *NBS1* gene [15, 16, 17] and we have recently found highly significant differences of the frequencies of the 5382insC mutation of the *BRCA1* gene, most prevalent in polish patients with familial breast/ovarian cancer [18], among consecutive breast cancer patients from East-Central, West-Central and South-East regions of Poland [19]. The small number of the 1100delC *CHEK2* mutation carriers found in the present study precludes a statistical evaluation of possible interregional differences of the frequency of this mutation among breast cancer patients from Poland. Our data, taken together with the results of the earlier study by CYBULSKI at al [7], who included however mainly patients from North-Western Poland, imply that the *CHEK2*1100delC* mutation contributes only very little to breast cancer burden in Poland.

Several authors have suggested that the 1100delC mutation may be more frequent among patients with familial breast cancer incidence. However with the exception of one patients with bilateral breast cancer, we found no carriers of this mutation among probands with one or more relatives with breast cancer or among probands with a second primary tumors at other site. This finding is not unexpected since recent studies suggest that the 1100delC mutation has a low penetration resulting only in a 2-3-fold increase breast cancer risk [7, 8]. It was however suggested that this risk may be much higher for some subtypes of breast cancer e.g. for contralateral tumors [6, 20]. Our findings are in line with this suggestion, although this subgroup of such patients in our study was still to small for definitive conclusions.

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