CHEK2-Positive Breast Cancers in Young Polish Women

Cezary Cybulski,1 Bohdan Górski,1 Tomasz Huzarski,1 Tomasz Byrski,1 Jacek Gronwald,1 Tadeusz Dębiak,1 Dominika Wokolorczyk,1 Anna Jakubowska,1 Elżbieta Kowalska,1 Oleg Oszurek,1 Steven A. Narod,2 and Jan Lubiriński1

Abstract

Purpose: To investigate the contribution of CHEK2 mutations to early-onset breast cancer in Poland and to establish the characteristic features of these cancers.

Experimental Design: We studied 3,228 women diagnosed with breast cancer under the age of 51 years and 5,496 population controls. CHEK2 mutations were detected by RFLP-PCR or allele-specific oligonucleotide-PCR assays. Clinical and pathologic features of CHEK2-positive cases and CHEK2-negative cases were compared.

Results: A truncating CHEK2 mutation (1100delC or IVS2+1G>A) was seen in 47 of 3,228 cases and in 34 of 5,496 controls (odds ratio, 2.4; P = 0.0001). The CHEK2 I157T missense mutation was present in 207 of 3,228 cases, compared with 264 of 5,496 controls (odds ratio, 1.4; P = 0.002). Breast cancers in women with a CHEK2 mutation were more commonly of lobular histology (21.5% versus 15.8%; P = 0.05), of size >2 cm (54.8% versus 43.5%; P = 0.01), or of multicentric origin (28.7% versus 19.5%; P = 0.01) than were cancers from women without a CHEK2 mutation. Bilateral cancers were equally common in both subgroups.

Conclusion: Three founder alleles in CHEK2 contribute to early-onset breast cancer in Poland. Breast tumors which arise in carriers of CHEK2 mutations seem to be similar to those of breast cancers in the population at large.

CHEK2 encodes the human analogue of yeast checkpoint kinases Cds1 and Rad53 (1, 2). Activation of these proteins in response to DNA damage prevents cellular entry into mitosis. A founder allele in CHEK2, 1100delC, has been reported to be a low-penetration breast cancer susceptibility allele in several studies, and in many ethnic groups (3–6). Other CHEK2 variants (IVS2+1G>A, I157T, and S428F) have also been suggested to confer increased breast cancer risks in different populations (7–9). Three CHEK2 founder mutations have been described in Poland (1100delC, IVS2+1G>A, and I157T) and one is present in the Ashkenazi Jewish population (S428F).

To investigate the contribution of CHEK2 mutations to early-onset breast cancer and to establish the characteristic features of these cancers, we studied 3,228 early-onset breast cancer cases and 5,496 population controls from Poland. Poland is well suited for this study because of the high frequency of CHEK2 founder mutations and the relative genetic homogeneity of the Polish population.

Materials and Methods

Patients. This study includes prospectively ascertained cases of invasive breast cancer diagnosed throughout Poland from 1996 to 2003. The study was initiated in Szczecin in 1996 and was extended to include Poznań in 1997 and Olsztyn in 1998. Fifteen additional centers began recruiting subjects in 2001. All patients diagnosed at age ≤50 years before 2003 at the affiliated hospitals were eligible. Patients with pure intraductal or intralobular cancer were excluded (ductal carcinoma in situ or lobular carcinoma in situ) but patients with ductal carcinoma in situ with microinvasion were included. Patients with sarcoma or lymphoma were excluded. Patients with a previous breast cancer were included, provided the cancer was in the opposite breast.

A total of 4,316 incident cases of invasive breast cancer were identified at the 18 different hospitals during the study period. Of these, 3,484 women accepted the invitation to participate (80.7%). Five patients withdrew their participation at a later time. A blood sample was obtained and DNA was successfully isolated from 3,474 cases. Among these, 198 women carried one of the three Polish founder BRCA1 mutations (4153delA, 5328insC, and C61G) and were excluded from the present study. These cases are described elsewhere (10). The medical record and pathology report were reviewed locally by the physician associated with the study and relevant information was forwarded to the study center in Szczecin. Information was recorded on age at diagnosis, stage, grade and lymph node status, estrogen-receptor status, multicentricity, and bilaterality. This information was collected without knowledge of the mutation status of the individuals. Women with a previous contralateral breast cancer or with a current diagnosis of bilateral cancer were considered to be bilateral. Patients with previous ipsilateral cancers were excluded. The study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin, Poland.

Pathology review. Tumor blocks and/or paraffin-embedded slides were requested from the corresponding pathology centers. One or more specimens were obtained from 3,136 of the 3,474 patients. A central pathology review was conducted in Szczecin by two pathologists.

Authors’ Affiliations: 1International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland and 2Centre for Research on Women’s Health, Toronto, Ontario, Canada.
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associated with the study. Pathologists were blinded to mutation status. Each case was reviewed with regard to histology (medullary, ductal, lobular, tubulo-lobular, other).

Mutation analysis. CHEK2 mutations were detected as previously described (11).

Population controls. To estimate the frequency of the Polish founder mutations in the general population, three control groups were combined. The first group consisted of 2,183 newborn children from 10 hospitals throughout Poland (Szczecin, Białystok, Gorzów, Katowice, Wrocław, Poznań, Opole, Łódź, and Rzeszów) between 2003 and 2006. Samples of cord blood from unselected infants were forwarded to the study center in Szczecin. The second control group was taken from healthy adult patients (1,079 women and 817 men) of three family doctors practicing in the Szczecin region. These individuals were selected randomly from the patient lists of family doctors. The third control group consisted of 1,417 young adults (705 women and 712 men) from Szczecin who submitted blood for paternity testing. There was no association between the allele frequencies and either age or sex in our control population. The frequency of a CHEK2 mutation was 5.6% in 1,529 adult men and 5.4% in 1,784 adult women. The frequency was 5.3% in 2,183 newborns, 5.5% in 2,056 controls between the ages 15 and 50 years, and 5.4% in 1,257 controls of ages >50 years.

Cancer history was available for 1,074 women from family doctors (age range, 15-91 years; mean 58.3 years). The frequency of a CHEK2 mutation in the 1,074 cancer-free women was 5.5% (5.0% for the I157F and 0.5% for a truncating variant).

Statistical analysis. The prevalence of the three CHEK2 alleles in cases and population controls was compared. Odds ratios (OR) were generated from two-by-two tables and statistical significance was assessed with the Fisher exact test. The ORs were used as estimates of relative risk.

Because of the potential for chemotherapy to alter tumor characteristics, women who received neoadjuvant chemotherapy were excluded from the analyses of histopathology type, size, nodal status, multifocality, and presence of estrogen receptors. However, they were retained for comparisons of bilaterality and family history.

Results

A CHEK2 mutation was identified in 252 of 3,228 women with breast cancer (7.8%), including I157T (207 times), IVS2+1G>A (31 times), and 1100delC (16 times). Overall, the OR for early-onset breast cancer in association with a CHEK2 mutation was 1.5 [95% confidence interval (95% CI), 1.2-1.8; Table 1]. The OR was higher (OR, 2.4; 95% CI, 1.5-3.7) for women with a truncating mutation (1100delC or IVS2+1G>A) than for women with a missense mutation (OR, 1.4; 95% CI, 1.1-1.6). The mean age of diagnosis in women with a CHEK2 mutation was similar to that of the noncarrier cases (Table 2). However, the mean age of diagnosis of women with a truncating mutation was 1.8 years lower than of women without a CHEK2 mutation (42.5 versus 44.3 years; P = 0.01).

The characteristics of the breast cancer cases in the 252 women with a CHEK2 mutation are presented in Table 2 and compared with noncarriers. The distribution of histologic types was similar in cases and controls. However, carriers of a CHEK2 mutation were slightly more likely to be diagnosed with lobular cancer than were noncarriers (21.5% versus 15.8%; P = 0.05). Intraductal cancers (ductal carcinoma in situ) with micro-invasion were also more common in women with a CHEK2 mutation than in noncarriers (11.3% versus 7.2%; P = 0.06). However, several comparisons were made and these differences were of marginal significance.

Carriers and noncarriers were similar with respect to tumor size, but tumors of sizes >2 cm were slightly more common among carriers of a CHEK2 mutation than in noncarriers (53.9% versus 43.5%; P = 0.01) and tumors of sizes <1 cm were less common in carriers compared with noncarriers (5.9% versus 11.2%; P = 0.05).

Carriers and noncarriers were similar with respect to estrogen receptor status (65.1% versus 63.7%; P = 0.8) and lymph-node status (45% positive versus 40.1%; P = 0.3). Bilateral tumors were equally common in both subgroups (2.3% versus 3.3%; P = 0.6). In contrast, CHEK2-associated cancers were significantly more likely to be multicentric (28.7%) than were tumors in noncarriers (19.5%; P = 0.01).

The great majority of the women with a CHEK2 mutation did not have a strong family of cancer—only 13.8% of the women with breast cancer with a CHEK2 mutation were from a family with two or more first-degree relatives with breast cancer. However, this was more frequent than reported by the noncarriers (8.9%) and the difference was statistically significant (OR, 1.6; P = 0.02).

Discussion

We identified a CHEK2 mutation in 7.8% of unselected early-onset breast cancer patients in Poland and have confirmed the contribution of three founder alleles to the burden of breast cancer. Overall, the tumors that arise in carriers of CHEK2 mutations seem to be similar to those of breast cancers in the population at large. However, carriers of CHEK2 mutation were slightly more likely to develop lobular breast cancer than noncarriers (21.8% versus 15.8%; P = 0.05). Previous analyses show that neither BRCA1 nor BRCA2 is associated with an increased frequency of lobular breast cancer (12, 13). However, increased frequency of lobular cancer was seen in non-BRCA1/2

<table>
<thead>
<tr>
<th>Cases (N = 3,228)</th>
<th>Controls (N = 5,496)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS2+1G&gt;A</td>
<td>31</td>
<td>22</td>
<td>2.4 (1.4-4.2)</td>
</tr>
<tr>
<td>1100delC</td>
<td>16</td>
<td>12</td>
<td>2.3 (1.1-4.8)</td>
</tr>
<tr>
<td>Any truncating</td>
<td>47</td>
<td>34</td>
<td>2.4 (1.5-3.7)</td>
</tr>
<tr>
<td>I157T</td>
<td>207</td>
<td>264</td>
<td>1.4 (1.1-1.6)</td>
</tr>
<tr>
<td>Total</td>
<td>252*</td>
<td>297*</td>
<td>1.5 (1.2-1.8)</td>
</tr>
</tbody>
</table>

*Two cases and one control had both truncating and missense mutations.
familial breast cancers, suggesting there may exist other breast cancer susceptibility genes that predispose specifically to lobular cancer (14). In addition, intraductal cancers (ductal carcinoma in situ) with microinvasion were more common in Polish women with a CHEK2 mutation than in noncarriers (11.3% versus 7.2%; \( P = 0.06 \)). In addition, CHEK2 mutation carriers were more likely to present with tumors >2 cm or to have tumors of multicentric origin than were noncarriers (\( P = 0.01 \)). However, we have made numerous statistical comparisons here and none of the associations was very strong. These data need to be confirmed in other studies.

A recent report from the Netherlands described breast cancers in 34 CHEK2 1100delC carriers and in 102 noncarriers. They found a significant difference between the two groups regarding steroid receptor status—carriers more frequently had ER-positive tumors (91% versus 69%; \( P = 0.03 \); ref. 15). In our larger group of CHEK2 mutation carriers, we saw a similar prevalence of ER-positive tumors (65.1%) as in noncarriers (63.7%).

A recent follow-up study reported that patients with the CHEK2 1100delC variant had a high risk of contralateral breast cancer (relative risk, 5.7; 95% CI, 1.7-19.7; ref. 15). This finding is supported by other studies that reported a six times higher prevalence of CHEK2 1100delC carriers among patients with bilateral breast cancer, as compared with patients with unilateral breast cancer (5, 16). Surprisingly, we saw a similar prevalence of bilateral cases among Polish women with a CHEK2 mutation (2.3%), compared with those without a CHEK2 mutation (3.3%), but the numbers were small and we restricted our analysis to early-onset cases.

A recent study from the United Kingdom suggested a multiplicative interaction between CHEK2 1100delC and other unknown susceptibility genes in women with a family history of bilateral breast cancer (17). Others have also suggested that CHEK2 might act in synergy with other cancer susceptibility genes (3, 5, 18). In this scenario, predisposition to bilateral breast cancer might be a result of the combined effect of a CHEK2 mutation and a mutation in a modifying gene. It is theoretically possible that the prevalence of modifying alleles might vary between the United Kingdom and Polish populations.

In previous studies, the CHEK2 1100delC mutation has been found in excess in familial breast cancer patients (3–5, 15). We found evidence for a higher prevalence of a CHEK2 mutation among cases with a first-degree relative affected with breast cancer than in cases with first-degree relative unaffected with breast cancer (OR, 1.6; 95% CI, 1.1-2.4; \( P = 0.02 \)); however, the effect was small and the great majority of patients with a CHEK2 mutation in our series of unselected breast cancer patients (86%) had no affected first-degree relative.

Our results confirm that the I157T variant is pathogenic for breast cancer. It was originally reported that the I157T variant does not seem to increase the risk of breast cancer (19, 20) and that CHEK2 protein carrying the I157T change has similar kinase activity, expression levels, and subcellular localization as endogenous CHEK2 (21). The I157T missense variant is localized in a functionally important domain of CHEK2 (the FHA domain) and protein with this mutation has been shown to be defective in its ability to bind and phosphorylate Cdc25A and to bind p53 and BRCA1 (22–24). The I157T protein may also have a dominant negative effect by forming heterodimers with wild-type CHEK2 (8). CHEK2 I157T variant has been reported to be associated with increased breast cancer risk in Finland, Germany, and Belarus (8, 25). This variant has been found to be associated with a susceptibility to other forms of breast cancer.

### Table 2. Comparison of CHEK2-positive and CHEK2-negative breast cancers cases

<table>
<thead>
<tr>
<th></th>
<th>CHEK2 mutation negative</th>
<th>CHEK2 mutation positive</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean), y</td>
<td>44.3</td>
<td>44.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Age group, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-30</td>
<td>1.7% (52 of 2,976)</td>
<td>3.6% (9 of 252)</td>
<td>0.07</td>
</tr>
<tr>
<td>31-40</td>
<td>17.2% (512 of 2,976)</td>
<td>15.9% (40 of 252)</td>
<td>0.7</td>
</tr>
<tr>
<td>41-50</td>
<td>81% (2,412 of 2,976)</td>
<td>80.5% (203 of 252)</td>
<td>0.9</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal, low grade</td>
<td>26.9% (622 of 2,315)</td>
<td>29.3% (54 of 186)</td>
<td>0.6</td>
</tr>
<tr>
<td>Ductal, high grade</td>
<td>12.7% (294 of 2,315)</td>
<td>10.7% (20 of 186)</td>
<td>0.5</td>
</tr>
<tr>
<td>Medullary</td>
<td>4.9% (113 of 2,315)</td>
<td>2.1% (4 of 186)</td>
<td>0.1</td>
</tr>
<tr>
<td>Lobular</td>
<td>15.8% (366 of 2,315)</td>
<td>21.5% (40 of 186)</td>
<td>0.05</td>
</tr>
<tr>
<td>Tubulo-lobar</td>
<td>3.6% (83 of 2,315)</td>
<td>4.3% (8 of 186)</td>
<td>0.8</td>
</tr>
<tr>
<td>Ductal carcinoma in situ</td>
<td>7.2% (168 of 2,315)</td>
<td>11.3% (21 of 186)</td>
<td>0.06</td>
</tr>
<tr>
<td>Other</td>
<td>5.1% (118 of 2,315)</td>
<td>4.8% (9 of 186)</td>
<td>1.0</td>
</tr>
<tr>
<td>Missing or unknown</td>
<td>23.8% (551 of 2,315)</td>
<td>16.7% (31 of 186)</td>
<td>0.03</td>
</tr>
<tr>
<td>Preoperative chemotherapy</td>
<td>24.5% (661 of 2,693)</td>
<td>27.7% (66 of 238)</td>
<td>0.3</td>
</tr>
<tr>
<td>ER-positive</td>
<td>63.7% (1,048 of 1,646)</td>
<td>65.1% (97 of 149)</td>
<td>0.8</td>
</tr>
<tr>
<td>Size, cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>11.2% (193 of 1,728)</td>
<td>5.9% (9 of 152)</td>
<td>0.05</td>
</tr>
<tr>
<td>1-2</td>
<td>45.3% (783 of 1,728)</td>
<td>40.1% (61 of 152)</td>
<td>0.2</td>
</tr>
<tr>
<td>&gt;2</td>
<td>43.5% (752 of 1,728)</td>
<td>53.9% (82 of 152)</td>
<td>0.01</td>
</tr>
<tr>
<td>Lymph nodes positive</td>
<td>40.1% (722 of 1,777)</td>
<td>45.0% (68 of 151)</td>
<td>0.3</td>
</tr>
<tr>
<td>Multicentric</td>
<td>19.5% (316 of 1,619)</td>
<td>28.7% (41 of 143)</td>
<td>0.01</td>
</tr>
<tr>
<td>Bilateral</td>
<td>3.3% (84 of 2,531)</td>
<td>2.3% (5 of 215)</td>
<td>0.6</td>
</tr>
<tr>
<td>Family history positive</td>
<td>8.9% (237 of 2,652)</td>
<td>13.8% (31 of 224)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

NOTE: For all comparisons, except age, bilaterality, and family history, the cases with preoperative chemotherapy are excluded. Family history refers to a first-degree relative affected with breast cancer.
cancer in Finland including prostate and colorectal cancers (26, 27). It has also been found to be associated with a susceptibility to chronic lymphocytic leukemia in the United Kingdom (28). In Poland, we saw a positive association between the I157T variant and breast, prostate, colon, kidney, and thyroid cancers (7), and recently, bladder, endometrial, and low-grade ovarian cancers.3

In conclusion, we estimate that ~8% of early-onset breast cancers arise in Poland in women with a germ line CHEK2 mutation. Both missense and truncating mutations seem to be pathogenic. The minority of mutation-positive cases have familial breast cancer. The tumors that arise in CHEK2 positive breast cancers in young women from Poland. Breast Cancer Res Treat. In press 2006. 11.

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3 Unpublished data.
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