Original Article

High frequency and allele-specific differences of \textit{BRCA1} founder mutations in breast cancer and ovarian cancer patients from Belarus


Breast cancer and ovarian cancer are common malignancies in Belarus accounting for about 3500 and 800 new cases per year, respectively. For breast cancer, the rates and age of onset appear to vary significantly in regions differentially affected by the Chernobyl accident. We assessed the frequency and distribution of three \textit{BRCA1} founder mutations 5382insC, 4153delA and Cys61Gly in two hospital-based series of 1945 unselected breast cancer patients and of 201 unselected ovarian cancer patients from Belarus as well as in 1019 healthy control females from the same population. Any of these mutations were identified in 4.4% of the breast cancer patients, 26.4% of the ovarian cancer patients and 0.5% of the controls. In the breast cancer patients, \textit{BRCA1} mutations were strongly associated with earlier age at diagnosis, with oestrogen receptor (ER) negative tumours and with a first-degree family history of breast cancer, although only 35% of the identified \textit{BRCA1} mutation carriers had such a family history. There were no marked differences in the regional distribution of \textit{BRCA1} mutations, so that the significant differences in age at diagnosis and family history of breast cancer patients from areas afflicted by the Chernobyl accident could not be explained by \textit{BRCA1}. We next observed a higher impact and a shifted mutational spectrum of \textit{BRCA1} in the series of Byelorussian ovarian cancer patients where the three founder mutations accounted for 26.4% (53/201). While the Cys61Gly mutation appeared underrepresented in ovarian cancer as compared with breast cancer cases from the same population (p = 0.01), the 4153delA mutation made a higher contribution to ovarian cancer than to breast cancer (p < 0.01). \textit{BRCA1} mutations were significantly enriched among ovarian cancer cases with a first-degree family history of breast or ovarian cancer, whereas the median age at ovarian cancer diagnosis was not different between mutation carriers and non-carriers. Taken together, these results identify three \textit{BRCA1} founder mutations as key components of inherited breast and ovarian cancer susceptibility in Belarus and might have implications for cancer prevention, treatment and genetic counselling in this population.

Key words: \textit{BRCA1} – breast cancer – founder mutations – genetic susceptibility – ovarian cancer – radiation

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Mutations in the breast cancer susceptibility gene \textit{BRCA1} contribute to a large fraction of familial breast and ovarian cancers although they are rare in the general population (1–3). \textit{BRCA1} mutations have also been associated with a distinct histology and high proliferation capacity of
breast tumours (4). On the other hand, the known functions of the \textit{BRCA1} gene product in DNA double-strand break repair have opened new therapeutic windows of opportunity in targeting just these pathways that may represent the Achilles’ heel of the repair-deficient tumour (5, 6). Detection of mutations in \textit{BRCA1} can be complex because of the large size of the gene and a marked allelic heterogeneity. But mutational screening can be economically accomplished in the presence of founder effects that result in the predominance of single mutations in some ethnically homogeneous populations.

Founder mutations in \textit{BRCA1} have been reported in Slavic and Baltic countries, with variable distribution throughout Eastern Europe (7–16). Frameshift mutations 5382insC in exon 20 and 4153delA in exon 11 and the RING finger domain missense substitution Cys61Gly in exon 5 have been reported as the most common \textit{BRCA1} mutations in these populations. Data about their incidence in the Byelorussian population, which is geographically placed between these countries, are scarce and until recently were based on only one small study, which included 19 families with hereditary breast-ovarian cancer syndrome from the Western part of Belarus (17).

In the first study presented here, we investigate a large breast cancer case-control series to estimate the potential impact of the three founder \textit{BRCA1} mutations on general breast cancer susceptibility and on regional differences that have been correlated with different breast cancer incidence ratios in Belarus (18). In a subsequent study, we show that \textit{BRCA1} mutations also account for a very high proportion of ovarian cancer cases in Belarus, and we provide some evidence for a differential distribution of the three founder mutations between breast and ovarian cancer in the same population.

\textbf{Materials and methods}

\textbf{Patients}

The Hannover-Minsk Breast Cancer Study (HMBCS) has been established during the past 7 years and has been a subject of previous genetic association studies of rare susceptibility alleles (19–22) as well as common polymorphisms (23, 24). Byelorussian cases were 1945 female breast cancer patients who had been diagnosed during the years 1998–2008 at the Byelorussian Institute for Oncology and Medical Radiology Aleksandrov N.N. in Minsk (n = 322) or at one of the regional oncology centres in Gomel (n = 459), Mogilev (n = 393), Grodno (n = 259), Brest (n = 286) or Vitebsk (n = 226), respectively. A total of 1266 cases (65.1%) were incident and 679 (34.9%) were prevalent. Median age at diagnosis was 48 years. Family history was available for 97.2% of the cases. As many as 305 patients (15.7%) reported that one or more first-degree relatives also had breast cancer, and 14 patients (0.7%) reported a first-degree relative with ovarian cancer; 98.7% of the cases had invasive breast cancer, and 71 patients (3.7%) had bilateral disease. Twenty-one breast cancer patients (1.1%) also had a personal history of ovarian cancer. Estrogen receptor (ER) status was available for 1217/1945 (62.6%) patients, and 267 (21.9%) had ER negative tumours.

The Hannover-Minsk Ovarian Cancer Study (HMOCS) has been established with the aim to investigate the molecular basis and identify genetic susceptibility alleles for ovarian cancer in the ethnically homogeneous population of Belarus. The hospital-based case series consists of 201 patients with epithelial ovarian cancer who had been ascertained during the years 2006–2009 at the Byelorussian Institute for Oncology and Medical Radiology Aleksandrov N.N. in Minsk, Belarus. Patients were not selected and there were no restrictions to enter the study, although we cannot exclude the possibility that younger women and women with family history of any cancer may have been more willing to participate. Median age at diagnosis was 50 years (age range: 24–76 years). Twelve patients (5.9%) reported that a first-degree relative also had ovarian cancer, while 10 patients (4.9%) reported a first-degree family history of breast cancer. One patient had a personal history of breast cancer, but she had not been part of the HMBCS series. Histology records were evaluated to confirm that all ovarian cancer patients had epithelial carcinoma and to identify histological subgroups. Records were fully informative for 98 patients. As many as 76 patients (78%) had serous, 14 (14%) had endometrioid, 7 (7%) had mucinous adenocarcinoma, and 1 (1%) had clear cell carcinoma. The other patients were recorded with adenocarcinoma only, with low-differentiated adenocarcinoma or with a mixed histology of adenocarcinoma, and one patient had a malignant Brenner tumour.

The Byelorussian controls were 1019 volunteers who had been ascertained during the same time period at the Institute for Inherited Diseases or at the Institute for Transfusion Medicine in Minsk, Belarus. Females were healthy at the time of recruitment and were excluded as controls if they reported a personal history of breast cancer. About 75% of controls consisted of women invited
for general medical examination at one of five regional gynaecology clinics (in Gomel, Mogilev, Grodno, Brest or Vitebsk) and of cancer-free volunteers ascertained at the Institute for Inherited Diseases in Minsk; 20% were cancer-free female blood bank donors recruited at Republic Blood Bank, placed in Institute for Transfusion Medicine, Minsk, Belarus; and the remaining 5% of controls were healthy cancer-free accompanists or in-law relatives of some breast cancer patients. Geographical regions of origin for cases and controls had a similar distribution. In total, 40.7% of the controls originated from contaminated areas, compared with 43.8% of the cases (Table 1). Median age among controls was 46 years (range 18–72 years). The control series had also been part of previous breast cancer genetic association studies (19–24).

The breast cancer patients and controls could be stratified by region of origin, taking into consideration that whole body doses accumulated over time after the Chernobyl accident in some of the study regions in South and Eastern Belarus (18). Patients were grouped in either non-contaminated living areas or contaminated living areas as defined and investigated by Pukkala and co-workers, with the levels of radiation exposure approximated from measurable ground contamination (18). The first group consisted of 1093 cases and 604 controls, the latter group consisted of 852 cases and 415 controls. Within the latter group, about one-third of cases were in the less-than 5-mSv category while 41 breast cancer cases originated from regions with an calculated annual cumulative dose ≥40 mSv (Table 1). The power of this stratification by geographical region was tested by comparing the ages at diagnosis of breast cancer and the proportion of patients with a positive family history between both groups using median and chi-squared tests (see Results).

Our study was carried out with informed consent of the probands and was approved by the Ethics Commission of the State Organization ‘Institute for Hereditary Diseases’, Ministry of Health, Republic of Belarus, and by the Institutional Review Board at Hannover Medical School, Hannover, Germany.

### Table 1. Geographical regions of origin of HMBCS cases and controls

<table>
<thead>
<tr>
<th>Series</th>
<th>Total (n)</th>
<th>Series</th>
<th>Total (n)</th>
</tr>
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<tbody>
<tr>
<td>Cases</td>
<td>1945</td>
<td>FH+veb</td>
<td>305</td>
</tr>
<tr>
<td>&lt;50 years</td>
<td>1072</td>
<td>Controls</td>
<td>1019</td>
</tr>
<tr>
<td>Not contaminated areas</td>
<td>1093</td>
<td>Not contaminated areas</td>
<td>604</td>
</tr>
<tr>
<td>FH+veb</td>
<td>137/1093</td>
<td>&lt;50 years</td>
<td>523</td>
</tr>
<tr>
<td>Minsk</td>
<td>322</td>
<td>Brest</td>
<td>286</td>
</tr>
<tr>
<td>Grodno</td>
<td>259</td>
<td>Grodno</td>
<td>104</td>
</tr>
<tr>
<td>Vitebsk</td>
<td>226</td>
<td>Vitebsk</td>
<td>108</td>
</tr>
<tr>
<td>Contaminated areas b (I–IV)</td>
<td>852</td>
<td>Contaminated areas b (I–IV)</td>
<td>415</td>
</tr>
<tr>
<td>FH+veb</td>
<td>168/852</td>
<td>&lt;50 years</td>
<td>549</td>
</tr>
<tr>
<td>Gomel</td>
<td>459</td>
<td>Gomel</td>
<td>207</td>
</tr>
<tr>
<td>Mogilev</td>
<td>393</td>
<td>Mogilev</td>
<td>208</td>
</tr>
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<table>
<thead>
<tr>
<th>Cases from contaminated areas (n)</th>
<th>Controls from contaminated areas (n)</th>
</tr>
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<tbody>
<tr>
<td>&lt;5 mSv 269/852</td>
<td>5–19.9 mSv 367/852</td>
</tr>
<tr>
<td>20–39.9 mSv 175/852</td>
<td>20–39.9 mSv 180/415</td>
</tr>
<tr>
<td>≥40 mSv 41/852</td>
<td>≥40 mSv 31/415</td>
</tr>
</tbody>
</table>

**a**Geographical distribution of cases and controls with respect to areas of differential ground contamination. Cases were also further stratified into groups with a first-degree family history of breast cancer (FH+ve), or with an age at diagnosis below 50 years (<50 years).

**b**Subset of patients with at least one first-degree relative affected with breast cancer.

**c**Regions contaminated with long-lived radionuclides after Chernobyl accident (18).
primer 5’-CCAAAGCGAGCAAGAGAATCTCTC-3’ (modified nucleotide position underlined) and the reverse primer 5’-GGGAATCCAAATTACAC AGC-3’. Thirty-five cycles of polymerase chain reaction (PCR) amplification were performed with annealing at 60°C, extension at 72°C and denaturation at 95°C using HotStar-Taq-Polymerase (Qiagen). PCR products were digested with DdeI or ScrFI (New England Biolabs), respectively, and the reaction products were separated on 3% agarose gels.

The two other BRCA1 mutations Cys61Gly and 4153delA (also known as c.T300G and c.4154delA, respectively) were investigated using allele-specific PCR assays. The Cys61Gly mutation was screened using a mutation-specific forward primer 5’-CCAGAAGAAAAGGCTTCACCTGGG-3’ within exon 5 in combination with the reverse primer 5’-CCTGTATAGGACGAGTAGTC-3’. Thirty-five cycles of PCR amplification were performed with annealing at 62°C, extension at 72°C and denaturation at 95°C using HotStar-Taq-Polymerase (Qiagen). In the presence of the Cys61Gly allele, a 221-bp PCR product was obtained. A 353 bp fragment of the MDC1 gene was used as an internal control for amplification. The 4153delA mutation was screened using a mutation-specific forward primer 5’-GGAA TTGGTTTCAGA TGA TCAG-3’ and reverse primer 5’-TCCTACTCAGATGTCAG-3’. Thirty-five cycles of PCR amplification were performed with annealing at 58°C, extension at 72°C and denaturation at 95°C using HotStar-Taq-Polymerase (Qiagen). In the presence of the 4153delA mutation, a 131-bp PCR product was obtained. A 269 bp fragment of the ATM gene was used as an internal control for amplification.

All reactions included at least one negative, one positive and one water control. Positive samples were verified by direct sequencing. For this purpose, exon 20 was amplified using the same primers as for 5382insC mutational screening, while exons 5 and 11 of the BRCA1 gene were amplified using the primers 5’-CTCTTTAAGGGCAGTTGTGAG-3’ (BR5i5) and 5’-TTCTCCTGTGGTGGCTTC-3’ (BR5i3); 5’-CTACTAGGGCATA GCACCCGGTC-3’ (BS5B) and 5’-CACCCTCATTAAATAGACTGGG-3’ (BR11R), respectively. Sequencing reactions were performed using the forward primer and BigDye v1.1 chemistry, were separated by capillary electrophoresis on a Genetic Analyzer 3100 Avant and were evaluated using Sequencing Analysis Software (Applied Biosystems, Foster City, CA, USA).

All statistical analyses were conducted using EpiCalc 2000 or Statistix 7.0 Analytical Software. The relative proportion of mutation carriers was derived from the ratio of the number of individuals observed with a given mutation and the total number of women tested. The odds ratio (OR) for breast cancer, given a mutation, was estimated as the cross-product of the 2 × 2 table, comparing cases and controls; these values and 95% confidence intervals (CIs) were obtained using EpiCalc 2000 software. Yates’ corrected chi-squared and p-values in 2 × 2 tables with two degrees of freedom were calculated using either Statistix 7.0 or EpiCalc 2000 software. Statistix 7.0 software was used for median tests to determine differences in the median age between groups.

Results
We assessed the frequency of the three previously reported BRCA1 mutations 5382insC, Cys61Gly and 4153delA in the frame of the HMBCS, with a hospital-based case-control series of 1945 breast cancer patients and 1019 healthy control females from Belarus. As listed in Table 2, we identified mutation 5382insC in 49 (2.5%), Cys61Gly in 20 (1.0%) and 4153delA in 17 (0.9%) of the cases. The 5382insC mutation was also found in one of 1019 Byelorussian population controls (0.1%), the Cys61Gly and 4153delA mutations were each identified in two control individuals (0.2%). Hence, a total of 4.4% of the cases and 0.5% of the controls were found to carry one of the three BRCA1 mutations. All mutation carriers were heterozygotes. These data established a high prevalence of the 5382insC mutation in Byelorussian breast cancer patients unselected for family history and confirmed a highly significant association with breast cancer (OR = 26.3, 95% CI = 3.6–190.8, p < 0.00001). Similarly, a significant association of the Cys61Gly missense mutation with breast cancer (OR = 5.3, 95% CI = 1.2–22.6, p = 0.02) and a borderline significant association of the 4153delA mutation with breast cancer (OR = 4.5, 95% CI = 1.0–19.4, p = 0.05) was confirmed.

Thirty-eight of the 49 breast cancer patients (78%) carrying the 5382insC allele had been diagnosed before age 50, and 19 patients (40%) reported a first-degree family history of breast cancer (family history was not available for two 5382insC cases). Two patients with 5382insC had bilateral disease and one had also ovarian cancer. 16 of the 20 breast cancer patients carrying the Cys61Gly mutation (80%) had been diagnosed below age 50, and 5 patients (25%) reported a
first-degree family history of breast cancer. One patient with Cys61Gly had bilateral disease. 13 of the 17 breast cancer patients carrying 4153delA allele had been diagnosed before age 50 (76%) and 6 patients reported a first-degree family history of breast cancer (38%, for one patient family history was not available). Also, none of the 4153delA breast cancer patients from Belarus. As expected for founder mutations in Byelorussian breast cancer patients, we noticed that the so-defined ages at first-degree family history of breast or ovarian cancer (FH) and controls are given as total numbers and relative percentages of the total patient series. Carriers are further stratified into groups -F H =< 50 years 13 (1.2%) — 6.2 (1.4; 27.7) p =< 0.01 -F H =< 50 years 11 (1.3%) — 6.7 (1.5; 30.1) p =< 0.01 4153delA < 50 years 17 (0.9%) 2 (0.2%) 4.5 (1.0; 19.4) p =< 0.05 < 50 years 6 (2.0%) — 10.2 (2.0; 50.8) p =< 0.01 < 50 years 8 (0.9%) — 4.8 (1.0; 22.8) p =< 0.06 < 50 years 25 (2.9%) — 30.8 (4.2; 227.6) p =< 0.00001 Cys61Gly < 50 years 20 (1.0%) 2 (0.2%) 5.3 (1.2; 22.6) p =< 0.02 < 50 years 2 (0.2%) — 8.5 (1.6; 44.0) p =< 0.01 5382insC < 50 years 19 (6.2%) — 68.0 (9.0; 507.4) p =< 0.00001 < 50 years 11 (1.3%) — 6.7 (1.5; 30.1) p =< 0.01 underscored areas 8 (0.9%) — 4.8 (1.0; 22.8) p =< 0.06 underscored areas 25 (2.9%) — 30.8 (4.2; 227.6) p =< 0.00001 BRCA1 (+/-) combined < 50 years 86 (4.4%) 5 (0.5%) 9.4 (3.8; 23.2) p =< 0.00001 < 50 years 44 (5.2%) — 11.0 (4.4; 28.0) p =< 0.00001 5382insC < 50 years 19 (6.2%) — 68.0 (9.0; 507.4) p =< 0.00001 underscored areas 8 (0.9%) — 4.8 (1.0; 22.8) p =< 0.06 underscored areas 25 (2.9%) — 30.8 (4.2; 227.6) p =< 0.00001<br><br>**The p-values are Yates' corrected calculated from 2 × 2 tables with 2df.**<ref><id>BRCA1</id> founder mutations were more common in the familial versus non-familial cases (9.8% versus 3.4%, p < 0.00001). The median age at onset of breast cancer was significantly lower for 5382insC mutation carriers compared with non-carriers (43 years versus 48 years, p < 0.001). The median age at onset of breast cancer in Cys61Gly or 4153delA carriers also appeared lower but was not significantly different from non-carriers (45.5 years and 44 years compared with 48 years). In total, the diagnosis of breast cancer was made earlier in the identified <ref>BRCA1</ref> mutation carriers compared with non-carriers. Among <ref>BRCA1</ref> mutation carriers, 45 of 63 patients with available ER status had ER-ve tumours (71.4%), indicating an about ninefold enrichment of mutation carriers among the 267 ER-ve patients (OR = 8.9, 95% CI = 5.1–15.6, p = 0.000001). The fraction of <ref>BRCA1</ref> mutation carriers with ER-ve tumours appeared slightly higher in the age group below 50 years (82%) than in patients diagnosed by age 50 or later (68%) but this difference was not significant (p = 0.3). We also asked whether a differential geographical distribution of <ref>BRCA1</ref> founder mutations may contribute to the regional differences in breast cancer incidence ratios that have been reported for Belarus (18). We stratified all patients by region of origin into two large groups according to different zones of ground exposition taking into account the increased average cumulative doses estimated for South and Eastern Belarus [(18), Table 1]. We noticed that the so-defined two patient groups differed in the median age at diagnosis of breast cancer which was earlier for the areas with higher ground exposition (50 years for patients in least contaminated regions versus 45 years in contaminated regions; p = 0.00001). The groups also differed in the proportion of familial cases which was higher in contaminated versus non-contaminated areas (OR = 1.71, 95% CI = 1.34–2.19, p < 0.00001). However, carrier frequencies for the three tested <ref>BRCA1</ref> mutations were not noticeably different between regions (5.2% vs. 3.8%, OR = 1.31, 95% CI = 0.85–2.02, p = 0.2). Furthermore, while the effect of carrying a <ref>BRCA1</ref> mutation on age at diagnosis was very pronounced in non-contaminated areas (median age 46 vs. 50 years, p < 0.00001), it appeared to
be less prominent in contaminated areas (42 vs. 45 years, p = 0.08).

The HMOCS investigates a hospital-based case-control series of 201 ovarian cancer patients from Minsk in Belarus. We also assessed the frequencies of the three BRCA1 mutations 5382insC, Cys61Gly and 4153delA in this series and compared them to the previously genotyped breast cancer and control series. As listed in Table 3, we identified mutation 5382insC in 26 (12.9%), the 4153delA mutation in 24 (11.9%), and the Cys61Gly mutation in 3 (1.5%) of the ovarian cancer patients. All mutation carriers were heterozygotes. Hence, the three founder BRCA1 mutations accounted for 26.4% (53/201) of the ovarian cancer cases and, by comparison with the control group, all three mutations were significantly associated with the presence of ovarian cancer (Table 3). A similar high OR was obtained when the control group was geographically restricted to the 246 females who originated from the Minsk region (OR = 87.7, 95% CI = 12.0–641.1, p < 0.00001). Interestingly, among the three mutations, the 4153delA mutation made a higher relative contribution in the ovarian cancer patient series (24/53) than in the previously analysed breast cancer series (17/86, p < 0.01). On the other hand, the Cys61Gly mutation was less predominant in the ovarian cancer series (3/53) than in the breast cancer study (20/86, p = 0.01).

A stratification of patients by family history revealed a BRCA1 mutation in 13/22 ovarian cancer patients (59.1%) with a first-degree history of breast or ovarian cancer, compared with 40/179 (19.9%) patients without such a family history (OR = 5.0, 95% CI = 2.0–12.6, p < 0.001). An assessment of tumour histology records revealed that the majority of BRCA1 mutation carriers had serous ovarian carcinomas (87%, compared with 78% of the total series), but the limited data set did not allow for more detailed analyses. The median age at diagnosis of ovarian cancer in BRCA1 mutation carriers was not significantly different from non-carriers (50 years compared with 51 years, p = 0.3). Similarly, the proportion of BRCA1 mutation carriers among patients diagnosed below the age of 45 was not different from the group with a later diagnosis (OR = 0.9, 95% CI = 0.5–1.8, p = 0.9). Hence, a positive family history rather than early-onset ovarian cancer was a characteristic for a BRCA1 founder mutation in our study.

Altogether, these results revealed a high proportion of BRCA1 founder mutations in unselected breast cancer patients from all over Belarus (4.4%), in a hospital-based series of ovarian cancer patients from Belarus (26.4%) as well as in a sample of healthy controls from the general Byelorussian population (0.5%).

### Discussion

We investigated three series of women from Belarus with breast cancer, ovarian cancer or healthy status for three founder mutations in the BRCA1 gene to gain more insight into the relevance of BRCA1 mutations for breast and/or ovarian cancer in this population and to provide a basis for future mutation-specific research and treatment strategies. The data from the breast cancer case-control study, HMBCS, indicated that about 1 in 22 breast cancer patients and 1 in 200 healthy women in Belarus may harbour a

<table>
<thead>
<tr>
<th>Table 3. BRCA1 founder mutations in Byelorussian ovarian cancer patientsa</th>
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<tbody>
<tr>
<td><strong>No. (%) of cases</strong></td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>5382insC</td>
</tr>
<tr>
<td>- FH+ve</td>
</tr>
<tr>
<td>- diagnosed &lt;45 years</td>
</tr>
<tr>
<td>Cys61Gly</td>
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<tr>
<td>- FH+ve</td>
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<tr>
<td>- diagnosed &lt;45 years</td>
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<tr>
<td>4153delA</td>
</tr>
<tr>
<td>- FH+ve</td>
</tr>
<tr>
<td>- diagnosed &lt;45 years</td>
</tr>
<tr>
<td>BRCA1 (+/-) combined</td>
</tr>
<tr>
<td>- FH+ve</td>
</tr>
<tr>
<td>- diagnosed &lt;45 years</td>
</tr>
</tbody>
</table>

aCarrier frequencies for the three BRCA1 founder mutations 5382insC, Cys61Gly, and 4153delA in Byelorussian ovarian cancer cases and controls are given as total numbers and relative percentages of the total patient series. Carriers are further stratified into groups with first-degree family history of breast or ovarian cancer (FH+ve), or with an age at diagnosis below 45 years (<45 years). Odds ratios are given for a comparison of cases versus all controls, or subgroups of cases versus all controls, respectively. Two-tailed Yates' corrected p-values are listed as calculated from 2 x 2 tables.
Bogdanova et al.

BRCA1 mutation. All three mutations, including the previously debated 4153delA mutation (10, 12, 13), were clearly associated with breast cancer risk. These mutations add to previously described breast cancer susceptibility alleles in the CHEK2, ATM and NBN genes that also show founder effects in the Byelorussian and in neighbouring Slavic populations (10, 19–22). Compared with those genes, ORs for the BRCA1 mutations were in a similar range or higher, with the highest value for 5382insC, albeit confidence limits were wide. BRCA1 mutations were strongly associated with earlier age at diagnosis, positive family history of breast cancer, and with ER negative tumours. The latter observation is in line with previous reports that BRCA1 mutant tumours, in particular those arising in young women, are mainly ER negative (26, 27).

A secondary but particular feature of breast cancer in Belarus relates to its reported association with environmental radiation exposure after the Chernobyl accident that took place in the year 1986 (18). This event has strongly affected the territory of Belarus adjacent to the Ukrainian border, and a more recent epidemiological study has provided evidence that women who resided in the most contaminated districts appear to have a two- to threefold increased risk of breast cancer compared with women in less contaminated areas (18). Furthermore, early data from the Byelorussian National Cancer Registry had provided evidence that breast cancer was diagnosed at a much younger age in afflicted zones such as the oblasts of Gomel and Mogilev (28), an observation that is clearly supported by our study. Our study also suggests that there is a higher rate of familial breast cancer in afflicted zones. Both findings could have an explanation by a higher occurrence of strongly predisposing genetic mutations as a result of regional founder effects. However, the distribution of the three BRCA1 mutations was not significantly different between regions in our study, and thus the observed interregional heterogeneity in terms of age at diagnosis and family history can presently not be explained as a consequence of strong genetic factors.

The ovarian cancer case-control study, HMOCS, indicates that about one in four ovarian cancer patients in Belarus may be heterozygous for a BRCA1 mutation. The high prevalence of BRCA1 mutations in ovarian cancer cases thus exceeds the already high prevalence of these mutations in Byelorussian breast cancer patients about fivefold. These observations are consistent with previous reports from other populations that also document a high proportion of BRCA1 founder mutations among ovarian cancer cases (9, 16, 29). While all three tested founder mutations were clearly associated with ovarian cancer, it is interesting to note that among these mutations the 4153delA mutation made a higher relative contribution in this patient series than in the previously analysed breast cancer series, whereas the Cys61Gly mutation was less predominant in our ovarian cancer series than in the breast cancer study. These observations indicate that there could be allele-specific differences in the expressivity of BRCA1 mutations in different organs. At present, there is no obvious biological explanation for such differences, but the same trend has been observed before in a family-based analysis of hereditary breast/ovarian cancer in Poland (30). In that study, the risk for breast cancer was higher among first-degree relatives of carriers of the Cys61Gly missense mutation compared to other mutations, and the risk for ovarian cancer was higher among first-degree relatives of carriers of the 4153delA mutation compared to other mutations (30).

The high prevalence of BRCA1 mutations in Belarus, one of the highest in the world, may be considered in future programmes for breast and ovarian cancer screening, cancer prevention and genetic counselling. There are also possible clinical implications in regard to treatment options and post-therapeutic surveillance. High response rates to platinum monotherapy have been reported for breast cancer patients carrying a BRCA1 mutation (5), and preliminary evidence indicates that this could extend to ovarian cancer patients with BRCA1 mutations as well (31, 32). Also, synthetic lethal approaches with PARP1 inhibitors such as olaparib have been used successfully in clinical trials of both breast and ovarian cancer patients including BRCA1 mutation carriers (6, 33). Such options may therefore play an increasingly important role in the future treatment and cure of this patient group. Furthermore, BRCA1 mutations have been associated with an increased risk of other cancers, and carriers therefore should be offered special surveillance and counselling regarding their disposition towards these malignancies. As early onset of disease was not a particular feature of BRCA1 mutation carriers among our ovarian cancer patients, and in view that more than half of the BRCA1 mutation carriers did not report a first-degree family history of breast or ovarian cancer, a major patient group would not receive such attention in the absence of mutation analysis. The high prevalence of three BRCA1 founder mutations may allow, in principle, to offer a direct mutational testing to all Byelorussian breast or ovarian cancer patients if accompanied by effective
means of counselling and therapeutic options; such a strategy has recently been discussed for early-onset breast cancer patients in the French-Canadian population (34).

Our hospital-based study aimed to investigate the distribution of founder *BRCA1* mutations in unselected breast or ovarian cancer patients from Belarus. About 1 in 22 breast cancer patients and about 1 in 4 ovarian cancer patients carried one of three *BRCA1* founder mutations. There appeared to be differences in the relative contribution of these mutations to breast or ovarian cancer, respectively. The high frequency of carriers may have important clinical consequences for early detection and tailored therapies to combat *BRCA1*-associated cancers in this population (35).

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**Conflict of interest**

Nothing to declare.

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