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High frequency of *BRCA1* 5382insC mutation in Russian breast cancer patients

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ABSTRACT

BRCA1 5382insC variant was repeatedly detected in Jewish breast cancer (BC) families residing in USA and Israel as well as in non-Jewish familial BC patients from Poland, Latvia, Hungary, Russia and some other European countries. However, the distribution of *BRCA1* 5382insC mutation in unselected BC cases vs. controls has been systematically investigated mainly in Ashkenazi Jews. Here we applied a case-control study design in order to evaluate the impact of *BRCA1* 5382insC allele on BC incidence in St Petersburg, Russia. High frequency of the *BRCA1* 5382insC allele was detected in a group of bilateral breast cancer patients (10.4%; 15/144). Randomly selected unilateral BC cases demonstrated noticeable occurrence of *BRCA1* 5382insC mutation as well (3.7%; 32/857), with evident excess of the carriers in the early-onset (⩽40 years) category (6.1%; 6/99) and in patients reporting breast and/or ovarian tumours in first-degree relatives (11.3%; 11/97). Strikingly, none of 478 middle-aged controls and 344 elderly tumour-free women carried the 5382insC variant. The presented data confirm a noticeable contribution of *BRCA1* 5382insC mutation in BC development in Russia, that may justify an extended *BRCA1* 5382insC testing within this population.

1. Introduction

Breast cancer (BC) is the most common neoplastic disease in women and affects approximately 1 out of 10 females. BC risk is significantly influenced by hereditary factors. Up to 5% of BC is attributed to the presence of germ-line mutation in *BRCA1* or *BRCA2* genes. Carriers of *BRCA* lesions have a highly elevated probability of breast and ovarian cancer development. *BRCA*-linked cancer cases are often associated with early onset, bilaterality, family history of breast and/or ovarian tumours as well as some specific histopathological and molecular features of neoplastic growth. Detection of *BRCA* mutations has significant clinical relevance, since it allows to identify women at risk who may benefit from intensive diagnostic monitoring and, in some circumstances, prophylactic surgery.[1–3]

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Comprehensive *BRCA* testing requires sequencing of the entire coding region of both *BRCA1* and *BRCA2* genes. This procedure remains too expensive even for the wealthiest countries in the world, and cannot be routinely applied outside Europe and North America. Fortunately, exhaustive *BRCA* sequencing can be avoided at least in some communities. For example, three so-called "founder" mutations (185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*) account for nearly all *BRCA* defects in Ashkenazi Jews. In Iceland, *BRCA2* 999del5 allele is the most common cause of *BRCA*-associated cancers. In these and some other ethnic groups, a straightforward detection of recurrent mutation by allele-specific PCR or other non-expensive techniques appears to be a reasonable and cost-efficient diagnostic approach.[4–6]

BRCA1 5382insC variant is frequently referred as a Jewish mutation. However, it has been also detected in Poland, Latvia, Hungary, Russia and some other European countries. Haplotyping analysis indicates that all 5382insC carriers have a common ancestor.[4,7,8] *BRCA1* 5382insC allele appears to be especially common in Russians, accounting for more than half (up to 94%) of *BRCA* mutations.[4,9–11] However, Russian studies on this mutation have been limited mainly by the analysis of a relatively small number of cancer families, and the prevalence of the *BRCA1* 5382insC variant in unselected BC series remains to be determined. In this study we have analyzed the significance of the *BRCA1* 5382insC mutation in Russia using a case-control study design.

2. Patients and methods

2.1. *Cases and controls*

Breast cancer patients from N.N. Petrov Institute of Oncology (St Petersburg, Russia) with histologically verified diagnosis were included in this study. 144 bilateral breast cancer cases (period of sampling: years 1984–2005) included 53 synchronous and 91 metachronous carcinomas. The mean patients age was 49.4 years for the onset of the first tumour (age range: 25–85 years), and 55.7 years for the time at diagnosis of the second one (age range: 28–87). The cohort of randomly collected incident unilateral BC consisted of 857 patients (mean age: 53.9; age range: 25–86; period of sampling: years 1996–2005). The female population standard (n = 478; mean age: 42.7; age range: 18–74; period of sampling: years 1998–2005) were recruited from blood donation volunteers (n = 400; age range: 18–54) as well as from cancer-free hospital controls (n = 78; age range: 55–74). Elderly tumour-free women (n = 344; mean age: 80.5; age range: 75–91; period of sampling: years 1998–2004) were collected from general hospitals of St Petersburg. The study was approved by the Ethics Committee of N.N. Petrov Institute of Oncology (St Petersburg). Other details on subject recruitment and DNA isolation have been described in our earlier reports.[12]

2.2. *Detection of BRCA1 5382insC mutation*

Carriers of *BRCA1* 5382insC variant were identified using real-time allele-specific PCR.[13] Primers were 5′-AAGCGAGCAAGAGAATTCCAG-3′ (specific for the wild-type allele), 5′-AGCGAGCAAGAGAATTCCCA-3′ (specific for the mutated allele), and 5′-AGAACCTGTGTGAAAGTATCTAGCACTG-3′ (common primer). The PCR mix included 50 ng genomic DNA or 1 μl of archival tissue lysate, 1 unit heat-activated Taq DNA polymerase, 1X PCR buffer (pH 8.3), 1.5 mM $MgCl_2$, 200 μM dNTP, 0.5 μM each primer, and 0.5X SYBR Green I in a final volume 10 μl. PCR amplification and product detection was carried out in iCycler iQ Real Time Detection System (Bio-Rad) for 50 cycles (95 °C for 35 sec., 65 °C for 60 sec., 72 °C for 60 sec.) after an initial activation of the polymerase at 95 °C for 10 min. Each amplification set included control wild-type and mutation-positive DNA samples. The specificity of the 168 bp PCR product was confirmed by melting curve analysis. All mutation-positive cases as well as some randomly selected DNA specimens were also subjected to conventional allele-specific PCR (35 cycles in the same conditions) followed by electrophoresis in 10% polyacrylamide gel.

3. Results

An example of *BRCA1* 5382insC genotyping is presented in the Fig. 1. Real-time PCR allowed a very straightforward discrimination between wild-type and mutant *BRCA1* alleles. In the 5382insC allele carriers, the difference between cycle thresholds (delta Ct) for intact and mutant alleles was always negligible, while in the non-carriers the amplification with the wild-type primer clearly surpassed the one with the mutation-specific oligonucleotide. The results of the real-time PCR detection were in complete agreement with the conventional gel-based allele-specific PCR analysis.

The results of *BRCA1* 5382insC detection in cases and controls are presented in the Table 1. The highest prevalence of the *BRCA1* 5382insC variant was detected in females affected by the bilateral form of breast cancer (10.4%; 15/144). Unilateral BC cases demonstrated a noticeable occurrence of *BRCA1* 5382insC mutation as well (3.7%; 32/857), with an evident excess of carriers in the early-onset (⩽40 years) category (6.1%; 6/99) and in patients reporting breast and/or ovarian tumours in first-degree relatives (11.3%; 11/97). Strikingly, none of 478 middle-aged controls and 344 elderly tumour-free women carried the 5382insC variant.

The probability of *BRCA* 5382insC presence clearly correlated with the number of clinical characteristics of familial BC (Table 2). BC patients with all three signs of hereditary cancer (bilaterality, early onset, and family history) demonstrated the highest prevalence of 5382insC mutation (25%; 3/12). This estimate approached to 15.6% (7/45) and 7.6% (20/263) in patients with two and one features of *BRCA* association, respectively. Importantly, in patients without any striking clinical presentation for *BRCA* mutation, many were 5382insC heterozygotes (2.6%; 17/652). As expected for the latter group, the highest frequency of *BRCA1* 5382insC mutation was observed in women aged 41–50 years (4.5%; 10/223); however, even relatively elderly patients (⩾61 years) had 1.3% (3/226) of 5382insC carriers.

4. Discussion

Our data indicate that *BRCA1* 5382insC mutation is responsible for approximately 1 out of 25 breast cancer cases in St Petersburg, Russia. The 5382insC variant was repeatedly

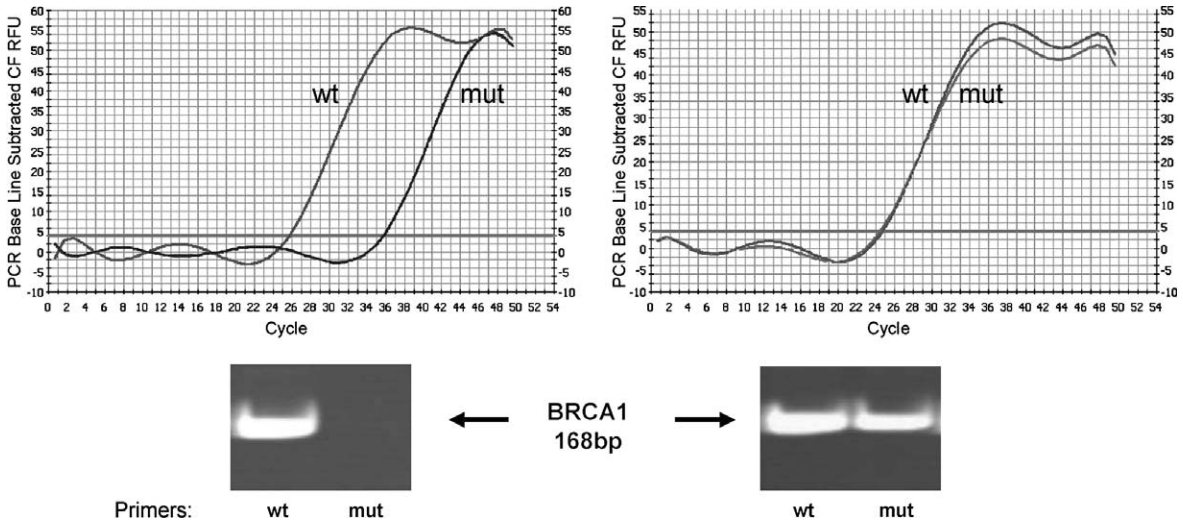

Fig. 1 – Allele-specific PCR detection of *BRCA1* 5382insC mutation. Normal DNA samples (left) demonstrate clear difference between cycle thresholds (delta Ct) upon real-time PCR analysis (top), and showed a single product after gel electrophoresis of the samples (bottom). In contrast, 5382insC heterozygotes (right) were characterized by simultaneous amplification of both wild-type and mutated alleles.

Table 1 – Occurrence of the BRCA1 5382insC mutation in patients with bilateral and unilateral breast cancer, healthy middle-aged female donors and elderly tumour-free women

Study groups	Number of the *BRCA1* 5382insC carriers (%)
Bilateral BC patients	15/144 (10.4%)
Age at diagnosis of the 1st tumour	
⩽40 years	7/33 (21.2%)
41–60 years	8/79 (10.1%)
⩾61 years	0/26 (0%)
Non-informative	0/6 (0%)
Family history[a]	
Positive	4/28 (14.3%)
Negative	11/112 (9.8%)
Non-informative	0/4 (0%)
Unilateral BC patients	32/857 (3.7%)
Age at diagnosis	
⩽40 years	6/99 (6.1%)
41–60 years	22/503 (4.4%)
⩾61 years	4/255 (1.6%)
Family history[a]	
Positive	11/97 (11.3%)
Negative	21/741 (2.8%)
Non-informative	0/19 (0%)
Healthy middle-aged female donors	0/478 (0%)
Elderly tumour-free women	0/344 (0%)

a Family history was defined as the presence of breast and/or ovarian cancer in mother or sister.

Table 2 – Number of clinical features of familial breast cancer and presence of BRCA1 5382insC

Features of familial BC	Number of the *BRCA1* 5382insC carriers (%)
3 features: bilaterality + early onset (⩽40 years) + family history[a]	3/12 (25.0%)
2 features	7/45 (15.6%)
Bilaterality + early onset (⩽40 years)	4/20 (20.0%)
Bilaterality + family history	1/16 (6.3%)
Early onset (⩽40 years) + family history	2/9 (22%)
1 feature	20/263 (7.6%)
Bilaterality	7/86 (8.1%)
Early onset (⩽40 years)	4/89 (4.5%)
Family history	9/88 (10.2%)
Absence of features of familial BC	17/652 (2.6%)
41–50 years	10/223 (4.5%)
51–60 years	4/203 (2.0%)
⩾61 years	3/226 (1.3%)

a Family history was defined as the presence of breast and/or ovarian cancer in mother or sister.

revealed in breast cancer families of different ethnicities residing in various European countries.[4] However, the distribution of *BRCA1* 5382insC mutation in unselected BC patients vs. controls has been systematically investigated mainly in Ashkenazi Jews (Table 3). Jewish studies revealed that the 5382insC allele accounted for 0.7–3.8% of BC cases, whereas its occurrence in healthy females was 0.1–0.3%.[14–20] Similar data were recently obtained from the Polish population, where the frequency of *BRCA1* 5382insC heterozygotes approached 2.1% in BC patients vs. 0.35% in controls.[21] In our data set, the prevalence of the 5382insC variant in BC patients is at the upper limit of variations described in the literature.[16,18,19,21] Furthermore, the analysis of bilateral breast cancer cases demonstrated 3-fold excess of the 5382insC allele in Russian vs. Jewish groups.[22] In contrast to other reports[14,15,17,21,23] we failed to detect 5382insC allele in non-affected subjects. However, our number of middle-aged controls is lower than in previous investigations. Absence of

Table 3 – BRCA1 5382insC mutation frequency in breast cancer patients and non-affected controls: comparison of different studies

Study	Country	Bilateral BC cases	Unselected BC cases	Healthy controls
Ashkenazi studies				
Roa[23]	USA			4/2717 (0.15%)[a]
Roa[23]	Israel			0/399 (0%)[a]
Struewing[14]	USA			11/3434 (0.32%)[c]
Fodor[15b]	USA			2/1715 (0.12%)[a,d]
Gershoni-Baruch[22]	Israel	2/55 (3.6%)		
Warner[16]	Canada		8/412 (1.9%)	
Bahar[17]	Australia			3/1068 (0.28%)[a]
Satagopan[18]	USA		4/573 (0.7%)	
Satagopan[18]	Canada		8/209 (3.8%)	
King[19]	USA		25/1008 (2.5%)	
Non-Ashkenazi studies				
Roa[23]	USA			0/1041 (0%)[a]
Backe[8]	Germany		8/800 (1.0%)	
Gorski[21]	Poland		43/2012 (2.1%)	14/4000 (0.35%)
Present study	Russia	15/144 (10.4%)	32/857 (3.7%)[e]	Middle-aged: 0/478 (0%); elderly: 0/344 (0%)[c]

a Males and females.
b Data on breast cancer patients are included in the report of Satagopan.[18]
c Females.
d Without family history of cancer.
e Bilateral cancers excluded.

the mutated allele in elderly females can be explained not only by the insufficient size of the group, but also by the age-related depletion of at-risk allele in healthy population.[24]

Overall it appears that the difference in the 5382insC allele frequencies between cases and controls is somewhat more pronounced in the present investigation when compared to other published data sets.[15,18,21] This may imply higher penetrance of the 5382insC variant in Russian subjects as compared to others, which can be attributed, for example, to distinct combinations of polymorphic modifier genes.[25] However, the limitations of our study have to be acknowledged. Most importantly, the collection of BC patients was hospital-based. It may that "unusual" BC patients, such as those with early onset and/or family history, are more likely to be forwarded for treatment to a specialized cancer research institute than "average" BC cases. If the latter is true, some overestimation of the role of *BRCA* defects is likely. On the other hand, the selection bias did not seem to be a confounding factor, since the proportion of early onset or family history-positive patients in our report did not exceed the one in other *BRCA1* 5382insC studies.[15,16,18]

As in other reports, *BRCA1* 5382insC variant demonstrated elevated occurrence in bilateral, early-onset, and familial cases (Tables 1 and 2). In accordance with literature data,[1,3] there was a positive correlation between the number of clinical signs of hereditary BC and the frequency of *BRCA1* 5382insC mutations, with the highest proportion of the heterozygote carriers in patients with all three above features, and the lowest frequency of the 5382insC in unilateral, late-onset, family history negative cases (Table 2).

Coupled to some other published studies, this report confirms frequent occurrence of *BRCA1* 5382insC variant in geographically distant regions of Russia, such as Moscow, St Petersburg, and Siberia.[9,10] The mere fact of the presence of a "founder" mutation in a multimillion nation spread from Baltic see to the Pacific Ocean is intriguing. The presented data may justify an extended *BRCA1* 5382insC testing in Russian population, with particular emphasis on early-onset, familial, and bilateral breast cancer cases.

Conflict of interest statement

Dmitry Trofimov is a Research Director of a small biotechnological company, which manufactures equipment, reagents, and kits for PCR laboratories. Other authors have no competing interests.

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