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Non-founder BRCA1 mutations in Russian breast cancer patients

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ABSTRACT

A few founder BRCA1 mutations (5382insC, 4154delA, 185delAG) account for up to 15% of high-risk (young-onset or familial or bilateral) breast cancer (BC) cases in Russia. The impact of non-founder BRCA1 mutations in this country is less studied; in particular, there are no reports analyzing gross rearrangements of this gene in the Russian patient series. We selected for the study 95 founder mutation negative high-risk BC cases. Combination of high-resolution melting (HRM) and sequencing revealed six presumably BC-associated alleles (2080delA, 4808C > G, 5214C > T, 5236G > A, 5460G > T, 5622C > T) and one variant of an unknown significance (4885G > A). The pathogenic role of the 5236G > A mutation leading to G1706E substitution was further confirmed by the loss of heterozygosity analysis of the corresponding tumor tissue. Multiplex ligation-dependent probe amplification (MLPA) revealed two additional BRCA1 heterozygotes, which carried BRCA1 deletions involving exons 1–2 and 3–7, respectively. Based on the results of this investigation and the review of prior Russian studies, three BRCA1 mutations (2080delA, 3819del5, 3875del4) were considered with respect to their possible founder effect and tested in the additional series of 210 high-risk BC patients; two BRCA heterozygotes (2080delA and 3819del5) were revealed. We conclude that the non-founder mutations constitute the minority of BRCA1 defects in Russia.

1. Introduction

A noticeable share of breast cancer (BC) predisposition is attributed to the presence of BC-associated germ-line mutations. BRCA1 and BRCA2 lesions have been detected in various races, ethnicities and geographic regions, while the impact of other genes (CHEK2, PALB2, NBS1) appears to be somewhat more population-specific [1,2]. Several countries and ethnicities demonstrate strong founder effect with respect to cancer genes [3]. For example, only three mutations (BRCA1 185delAG, BRCA1 5382insC and BRCA2 6174delT) account for the most of BRCA mutations in Ashkenazi Jews, while the BRCA2 999del5 serves as a major cause of hereditary breast–ovarian cancers in Iceland. Somewhat surprisingly, strong founder effect is also observed in a huge territory of Eurasia populated by people of Slavic descent, including Poland, Russia, Belarus and possibly some other countries. BRCA1 5382insC allele accounts for more than a half of all known BC-associated mutations in the area ranging from the Baltic Sea to the Pacific Ocean, while the contribution of other recurrent genetic lesions (BRCA1 4154delA (frequently quoted as

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BRCA1 4153delA), BRCA1 185delAG, CHEK2 1100delC, CHEK2 IVS2 + 1G > A, NBS1 657del5) varies from region to region [4–11].

We have shown previously that BRCA1 founder mutations (5382insC, 4154delA and 185delAG) are frequently detected in so-called high-risk BC cases, i.e. in patients demonstrating clinical signs of familial cancer syndrome (early onset or family history or tumor bilaterality) [10]. It remains unknown, if there are some other founder BRCA1 mutations in Russia, and whether non-founder mutations also constitute a significant share of BRCA1 defects in this population. Furthermore, gross gene alterations constitute a noticeable portion of BRCA1 mutations in some countries and ethnicities [1], however, their relevance to Russian BC patients has not been investigated yet. The present study was aimed to address the above questions. We initially subjected 95 high-risk BC patients to the comprehensive analysis of BRCA1 small sequence alterations and large genomic rearrangements. We then pooled the results of our investigation and the available literature data, and identified three novel mutations with potential founder effect; these mutations have been tested in an independent panel of 210 genetically enriched BC cases.

2. Materials and methods

We initially considered 200 blood-derived DNA samples from BC patients, who were treated in N.N. Petrov Institute of Oncology (St.-Petersburg) between years 1998 and 2006, and bore at least one clinical sign of hereditary cancer (age $\leqslant$ 40 years, or BC or ovarian cancer in mother or sister, or bilateral form of the disease); 154 of these cases have already been described in our previous report [10]. Genetic testing for the presence of founder mutations (BRCA1 5382insC, BRCA1 4154delA, BRCA1 185delAG, BRCA2 6174delT, CHEK2 1100delC, CHEK2 IVS2 + 1G > A, NBS1 657del5) has been performed. Forty samples had one of the above germ-line mutations (BRCA1 5382insC: 26; BRCA1 4154delA: 2; BRCA1 185delAG: 2; BRCA2 6174delT: 1; CHEK2 1100delC: 5; CHEK2 IVS2 + 1G > A: 2; NBS1 657del5: 2). Ninety-five of the remaining 160 DNA samples, which contained the largest amount of DNA, were selected for the full-length BRCA1 analysis. Eighty-eight out of these 95 patients had a single clinical feature of hereditary BC (early onset: 58; affected first-degree relative(s): 18; bilaterality: 12); seven remaining women had a combination of two clinical indicators of BC predisposition (early onset and affected first-degree relative(s): 6; bilaterality and affected first-degree relative(s): 1). BRCA1 testing included identification of micromutations and large genomic rearrangements. Screen for micromutations was performed using high-resolution melting (HRM) analysis followed by single nucleotide polymorphism (SNP) genotyping or sequencing of suspicious fragments; all methodological details for this laboratory procedure have been comprehensively described in [12]. All detected mutations were designated according to the commonly used nomenclature (Breast Information Core (BIC) database, http://research.nhgri.nih.gov/bic/); the corresponding Human Genome Variation Society (HGVS) identifiers are presented in the Table 1 [33; see also http://www.hgvs.org/mutnomen/ for the update]. Detection of large rearrangements of BRCA1 gene was done by multiplex ligation-dependent probe amplification (MLPA) using P002 kit for the initial screening and P087 kit for the validation of revealed abnormalities; the exact description of the MLPA protocol is presented in the web site of the kits manufacturer (MRC Holland, http://www.mrc-holland.com).

Those BRCA1 mutations, which were detected at least twice in Russian studies [16,34–37; present report] were subjected to the additional testing in the enlarged set of young-onset or familial or bilateral BC samples (n = 210;

Table 1
Non-founder DNA alterations in Russian breast cancer patients.

Sample	Genetic variant	Consequence	Clinical importance	Described in other countries/ethnicities	Number or submissions to the BIC database by December, 2009
1083	2080delA (c.1961delA)	K654SfsX47 (frameshift)	Deleterious	Canada (English) [13], Japan [14], Korea [15], Russia [16], Italy [17], Spain [18], USA (Dutch) [19]	31
498	4808C > G (c.4689C > G)	Y1563X (nonsense)	Deleterious	Germany [20], USA (Irish) [19], USA [21]	51
1148	4885G > A (c.4766G > A)	R1589H (missense)	Neutral (?)	Submitted by the Myriad Genetics Inc. to the Breast Information Core database	2
276	5214C > T (c.5095C > T)	R1699 W (missense)	Deleterious	Germany [22], Sweden [23]	13
566	5236G > A (c.5117G > A)	G1706E (missense)	Deleterious (?)	Spain [24]	7
360	5460G > T (c.5341G > T)	E1781X (nonsense)	Deleterious	–	–
1100	5622C > T (c.5503C > T)	R1835X (nonsense)	Deleterious	Germany [25], Pakistan [26], Philippines [27], the Netherlands [Breast Information Core database]	63
178	Deletion of the exons 1 and 2		Deleterious	Czech Republic [28], Germany [29], Italy [30], Poland [31]	Not applicable
990	Deletion of the exons 3–7		Deleterious	UK [32]	Not applicable

Point mutations are designated according to the commonly used nomenclature (Breast Information Core (BIC) database, http://research.nhgri.nih.gov/bic/). The Human Genome Variation Society (HGVS) identifiers are presented in brackets [33; see also http://www.hgvs.org/mutnomen/ for the update]. The 2080delA mutation, which corresponds to the last nucleotide in polyA(8) track, is designated as 2073delA in [16].

all negative for the most common Russian BC-predisposing mutations, i.e. BRCA1 5382insC, BRCA1 4154delA and CHEK2 1100delC [10]). BRCA1 2080delA allele was screened by HRM/sequencing analysis [12]. The 3819del5 and 3875del4 variants were analyzed by polyacrylamide gel electrophoresis using primers AGAGCTTCCCTGCTTCCAAC and GTAGCAACGGTGCTATGCCT for the former, and TCAGTCTACTAGGCATAGCACC and CAATATTACCTGGTTACTGCAGTC for the latter mutation, respectively.

The detection of the loss of heterozygosity (LOH) in the BRCA1 locus in the tumor sample carrying the 5236G > A mutation was done using allele-specific primers CGGACACTGAAATATTTTCTAGG and CGGACACTGAAATATTTTCTAGA, and the common primer TAGGTGTAAAAATGCAATTCTGA, as described in Suspitsin et al. [38].

3. Results

HRM/sequencing analysis of 95 high-risk founder mutation-negative BC cases revealed seven samples with BRCA1 point mutations (Table 1). Six of these mutations have been already reported by other investigators, while the mutation 5460G > T appears to be novel. Four of seven identified BRCA1 sequence alterations (2080delA, 4808C > G, 5460G > T and 5622C > T) lead to the frameshift or stop-codon and therefore are clearly pathogenic. The 5214C > T missense mutation affecting codon 1699 is also classified by the Breast Information Core (BIC) database (http://research.nhgri.nih.gov/bic/) as BC-predisposing mutation, based on ample laboratory and clinical evidence.

Two other missense mutations, 4885G > A and 5236G > A, are classified by the BIC resource as variants with an unknown significance. Tumors triggered by the germ-line BRCA1 defect are frequently characterized by somatic deletion of the remaining (wild-type) allele of the gene, therefore loss of heterozygosity (LOH) analysis is regarded as an efficient tool to discriminate between pathogenic and neutral nucleotide substitutions [39]. The corresponding tumor tissue was available only from the patient carrying the 5236G > A variant; the LOH test revealed an unambiguous somatic loss of the intact BRCA1 allele (Fig. 1).

For the MLPA study we considered 89 remaining DNA samples, which did not contain deleterious micromutations (Table 1). Eighty-five specimens had a sufficient quality of DNA for the detection of large genetic alterations. The MPLA test identified two tumors with the BRCA1 inactivation; both these mutations have been already reported previously (Table 1).

Based on results of the present study and the available literature data [16,34–37], three mutations may deserve testing with respect to possible founder effect (Tables 1 and 2). One of the mutations identified in our patient series, 2080delA, has been previously described in Russian ovarian cancer family [16] and is mentioned in subsequent manuscripts of the same group of authors [35,37]. Another variant, 3819del5, has been detected in two unrelated patients in the study of Grudinina et al. [36]. Carriers of the 3875del4 allele were identified by Gayther et al. [16] and Grudinina et al. [36]. We undertook the analysis of the three mentioned mutations in the additional series of 210 high-risk, founder mutation-negative BC cases, and revealed two heterozygous patients carrying the 2080delA and the 3819del5 variants, respectively.

4. Discussion

This is so far the largest BRCA1 study performed on Russian high-risk BC patients, and the first report assessing contribution of gross rearrangements of the gene in BRCA1 mutational spectrum in Russia. It confirms that non-founder mutations constitute the minority of BRCA1 defects in this country. Indeed, the screening for three recurrent BRCA1 mutations (5382insC, 4154delA and 185delAG) in the initial set of 200 high-risk BC patients led to the identification of 30 (15%) carriers. However, when 95 of the remaining founder mutation negative cases were subjected to the comprehensive BRCA1 analysis, only eight clearly pathological variants were identified. These data are consistent with other Russian studies [34,35,37].

Importantly, a single deleterious variant, the 5382insC, is identified in more than a half of all BRCA1 mutation carriers in Russia. Given the high frequency of this mutation in non-selected BC cases (3.7% [40]), one would argue for an extended application of BRCA1 5382insC test in the clinical setting. There are several other BRCA1 deleterious alleles which demonstrate a trend towards non-random occurrence in Russia (4154delA, 185delAG, 2080delA, 3819del5), however, their frequency does not exceed 1% each even in genetically enriched categories of BC patients. If we consider all published studies, which involved full-length BRCA1 sequencing (Table 2), the total number of tested high-risk patients approaches 333; this provides 85% probability of detecting at least two cases with mutation occurring at a frequency of 1%. One may conclude that the list of Russian founder BRCA1 mutations is close to its completion.

This report added to the evidence for pathogenic significance of the 5236G > A substitution, as the LOH analysis identified a somatic loss of the remaining (wild-type) BRCA1 allele in the corresponding tumor tissue. It should be commented, that a caution must be taken while inter-

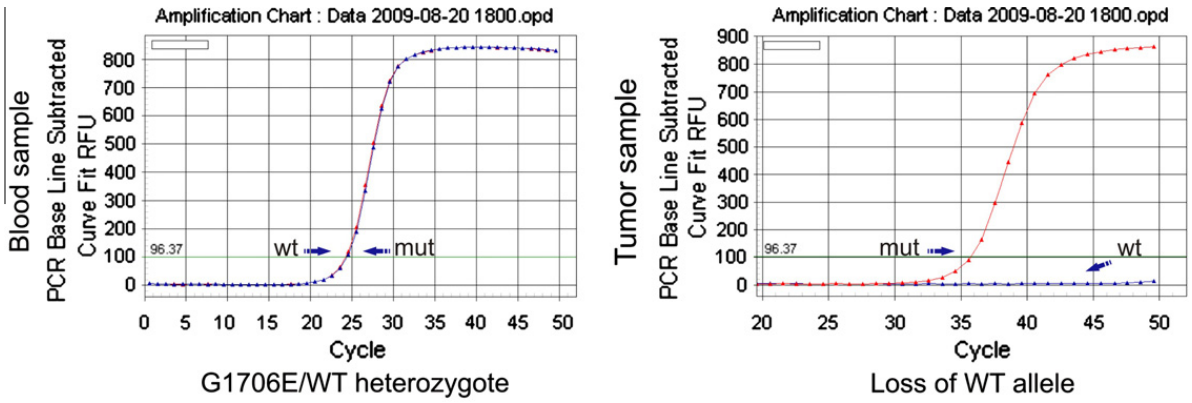

Fig. 1. Loss of the wild-type BRCA1 allele in the tumor obtained from the G1706E mutation carrier. Real-time PCR demonstrates simultaneous amplification of both intact and mutated allele in the blood DNA, while only the latter is amplified in the tumor sample.

Table 2
Sequencing analysis of BRCA1 gene in Russian patients.

Study	Subjects	Founder mutations	Non-founder mutations	Comments
Gayther et al. [16]	19 families with at least two first-degree relatives affected by ovarian cancer (Moscow and some other regions of Russia)	5382insC (9), 4154delA (3)	2080delA (1), 3875del4 (1)	
Tereschenko et al. [34]	25 breast/ovarian cancer families, 22 patients $\leqslant$ 40 years, six bilateral BC patients (Siberia)	5382insC (3)	None	BRCA2 analyzed as well; four mutations identified
Loginova et al. [35]	49 patients with familial breast cancer (Moscow)	5382insC (15), 185delAG (1)	None	
Grudinina et al. [36]	43 high-risk BC patients (St.-Petersburg)	5382insC (4)	2274insA (1), 2963del10 (1), 3726C > T (1), 3819del5 (2), 3875del4 (1)	
Smirnova et al. [37]	74 ovarian cancer patients (Moscow)	5382insC (7), 4154delA (1), 185delAG (1), 300T > G (2)	2080delA (1)	BRCA2 exon 11 analyzed as well; no deleterious mutations identified
Present study	95 high-risk BC patients, carriers of founder mutations excluded	Not applicable	2080delA (1), 4808C > G (1), 5214C > T (1), 5236G > A (1), 5460G > T (1), 5622C > T (1), deletion of the exons 1 and 2 (1), deletion of the exons 3–7 (1)	Large gene rearrangements considered

The number of subjects with mutation is indicated in brackets. BRCA1 mutations are designated according to the commonly used BIC nomenclature. Their corresponding HGVS designations are: 185delAG = c.68_69delAG, 300T > G = c.181T > G, 2080delA = c.1961delA, 2274insA = c.2157_2158insA, 2963del10 = c.2844_2853del10, 3726C > T = c.3607C > T, 3819del5 = c.3700_3704delGTAAA, 3875del4 = c.3756_3759delGTCT, 4154delA = c.4034delA, 4808C > G = c.4689C > G, 5214C > T = c.5095C > T, 5236G > A = c.5117G > A, 5382insC = c.5266dupC, 5460G > T = c.5341G > T, 5622C > T = c.5503C > T [33; see also http://www.hgvs.org/mutnomen/ for the update]. BRCA1 4154delA mutation (BIC nomenclature) is frequently quoted as BRCA1 4153delA in the literature [1].

preting the LOH data; some studies argue that the LOH analysis has limited power to discriminate between BC-predisposing and neutral BRCA1 variants [41,42]. The 5236G > A mutation results in the replacement of glycine by glutamine in the codon 1706. BIC database still refers to the unknown clinical importance of the G1706E mutation. However, there is a number of research communications providing arguments for the deleterious impact of the 5236G > A allele: in addition to the preferential somatic inactivation of the wild-type allele, this substitution co-segregates with the disease in cancer families, affects an evolutionary conservative aminoacid site, modifies *in vitro* transcriptional activity of the protein, and is likely to influence conformation of the BRCA1 molecule [24,43–47]. Based on the literature data, this variant is classified as pathogenic by a more recent online resource, the Leiden Open Variation Database (LOVD) (http://chromium.liacs.nl/LOVD2/cancer/home.php). Another missense mutation, 4885G > A, leading to the substitution of arginine by histidine in the position 1589, is characterized both by BIC and LOVD databases as the variant with an unknown significance. Some indirect evidence suggests neutral role of this mutation in determining BC risk [47].

While the picture of BRCA1 mutations in Russia is getting clear, little has been done for the analysis of BRCA2 gene alterations (Table 2). Studies from the neighboring country, Poland, demonstrate negligible impact of this gene in Slavic familial breast/ovarian cancers [4]. Smirnova et al. [37] analyzed exon 11 of the BRCA2 gene in 74 ovarian cancer patients from Moscow, and detected no deleterious alterations. However, in the study of high-risk BC cases from Russian Siberia, the frequency of BRCA2 mutations exceeded the one for BRCA1 gene [34]. Therefore, the comprehensive study on the impact of the BRCA2 alterations in breast/ovarian cancer in Russia is highly warranted.

Until recently, the efforts for the identification of BRCA1/2 carriers were aimed mainly at the identification of women-at-risk. Given the contemporary medical realities in Russia, such as shortage of funds and insufficient acceptance of preventive interventions, large-scale application of the comprehensive BRCA1 testing may be considered premature; instead, introduction of a panel of simple and non-expensive founder mutation tests may be viewed as a good compromise for the time being [10]. Furthermore, our earlier study has demonstrated that as many as 1.3% BC patients without any clinical signs of hereditary BC (i.e. unilateral BC cases aged above 60 years and reporting no family history of the disease) carry BRCA1 5382insC mutation; probably, this mutation deserves to be analyzed in all women with BC diagnosis [40]. BRCA1 testing may soon gain a different focus, given recent data on exceptional sensitivity of BRCA1-related cancers to cisplatin monotherapy [48,49] or PARP inhibitors [50]. When applied for the choice of anticancer drugs, BRCA1 analysis is likely to provide immediate health and cost benefits, and therefore be recommended for extended application.

Conflict of interest

None declared.

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