

BRCA1 4153delA founder mutation in Russian ovarian cancer patients

Nadezhda Yu Krylova, Oksana S Lobeiko, Anna P Sokolenko, Aglaya G Iyevleva, Maxim E Rozanov, Natalia V Mitiushkina, Madina M Gergova, Tatiana V Porhanova, Adel F Urmancheyeva, Sergey Ya Maximov, Alexandr V Togo, Evgeny N Imyanitov

NN Petrov Institute of Oncology, St. Petersburg, Russia; Medical Academy of Postgraduate Studies, St. Petersburg, Russia

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Corresponding author: Evgeny N Imyanitov, NN Petrov Institute of Oncology, Pesochny-2, 197758 St. Petersburg, Russia, phone +78 12 596 89 21, fax +78 12 596 89 47, Email: evgeny@imyanitov.spb.ru

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Abstract

The BRCA1 4153delA allele is frequently referred to as the Russian founder mutation, as it was initially detected in several cancer families from Moscow. Our earlier studies have demonstrated 1% occurrence of BRCA1 4153delA heterozygosity in familial and/or early-onset and/or bilateral Russian breast cancer (BC) patients. Since literature data suggest that the 4153delA variant is more associated with ovarian cancer (OC) than with BC, we expected to reveal a highly elevated frequency of this genotype in Russian ovarian cancer series. However, real-time allele-specific PCR genotyping has detected only two BRCA1 4153delA carriers out of 177 unselected OC patients (1.1%). Both these carriers were early-onset and had serous carcinomas of grade 3. Thus, our study supports neither the Russian origin of BRCA1 4153delA mutation, nor its selectivity towards ovarian versus breast cancer predisposition.

Introduction

Ovarian cancer (OC) affects approximately 1 out of 70 women during their lifetime and is regarded as the most lethal gynaecological malignancy. Early ovarian cancer does not usually cause symptoms; therefore it can be detected only through prophylactic medical check. Furthermore, even the combination of sophisticated technologies, such as ultrasound examination, magnetic resonance imaging, and CA-125 antigen measurement, does not fully guarantee timely OC diagnosis [1-3]. A significant portion of OC cases arise due to the presence of a germline mutation in the BRCA1 or BRCA2 gene. Estimates of occurrence of BRCA mutations in unselected OC cases vary from 3% to 35% (reviewed in [4]). Surprisingly, the occurrence of BRCA defects in random series of OC is similar to that observed in high-risk categories of breast cancer (BC), such as familial

and/or early-onset and/or bilateral cases of the disease. Therefore, the mere fact of ovarian cancer diagnosis appears to justify BRCA testing.

Complete analysis of BRCA1 and BRCA2 genes cannot be used yet on a large scale due to the high cost of appropriate laboratory procedures. Fortunately, in some ethnic groups and geographic regions the BRCA mutation spectrum is limited to a small number of so-called founder mutations [5]. For example, only a few simple PCR tests allow most of the BRCA carriers in Israel, Iceland, Poland, Russia, etc to be revealed. One of these founder mutations is BRCA1 4153delA, which was originally described in Russian cancer families [6]. This mutation also occurs in Poland, Latvia, Lithuania and Belarus [7-13]. Some evidence suggest that this mutation is more associated with ovarian than with breast cancer. In particular, the initial study of Gayther et al. [6], which identified three 4153delA mutations in 19 families, was

specifically focused on ovarian cancer pedigrees. Furthermore, a series of investigations performed by Lubinski and associates demonstrated a noticeable occurrence of BRCA1 4153delA in ovarian but not in breast cancer patients. For example, a study of unselected Polish patient series has revealed 8/364 (2.2%) carriers in OC, but only 1/2012 (0.05%) in BC cases [4, 11]. We have recently tested 302 breast cancer patients characterized by family history and/or early-onset and/or bilaterality, and detected 3 (1%) 4153delA allele carriers [Sokolenko et al., manuscript submitted]. Based on the apparent Russian origin of this mutation [5, 6] as well as on its probable specificity towards OC predisposition [4, 11, 14], we hypothesized that unselected ovarian cancer cases from Russia will be characterized by highly elevated occurrence of the 4153delA variant.

Materials and methods

Patients and DNA isolation

The study included 177 ovarian cancer patients, who underwent surgical treatment in N.N. Petrov Institute of Oncology, St. Petersburg, Russia. Patients' characteristics are described in Table 1. Archived

Table 1. Clinical characteristics of ovarian carcinoma patients

Mean age	52.2 years
Age range	21-87 years
Age distribution	
≤40 years	24 (13.6%)
41-60 years	104 (58.8%)
≥61 years	48 (27.1%)
Non-informative	1 (0.6%)
T status	
T=1	24 (13.6%)
T>1	151 (85.3%)
Non-informative	2 (1.1%)
N status	
N=0	61 (34.5%)
N=1	44 (24.9%)
Nx	70 (39.5%)
Non-informative	2 (1.1%)
Tumour differentiation	
Grade 1	19 (10.7%)
Grade 2	47 (26.6%)
Grade 3	94 (53.1%)
Non-informative	17 (9.6%)
Histology	
Serous adenocarcinoma	144 (81.4%)
Mucinous adenocarcinoma	6 (3.4%)
Adenocarcinoma, unspecified	21 (11.9%)
Other	6 (3.4%)
Total	177 (100%)

specimens of the excised normal tissues were used as a source of DNA. DNA isolation was performed as described in [15]. Briefly, tissue sections were deparaffinized in 2 changes of xylene, and then boiled for 5 min. in 100 μ l of the lysis buffer (10 mM Tris-HCl, pH 8.3; 1 mM EDTA; 0.5% NP-40, 0.5% Tween 20). Proteinase K was subsequently added up to the concentration of 500 μ g/ml. Protein digestion was done overnight at 60°C. Next, the tissue lysates were boiled again in order to inactivate proteinase K. Finally, samples were diluted to 1:10 with water in order to decrease the concentration of PCR inhibitors.

BRCA1 4153delA genotyping

BRCA1 4153delA was detected using SYBR Green based real-time allele-specific PCR. Primers were 5' -GACTGCAAATACAAACACCCA - 3' (common), 5' -AGCCCGTTCCTCTTTCTTC - 3' (specific for the wild-type allele), and 5' -AGCCCGTTCCTCTTTCTCA - 3' (specific for the 4153delA mutation). PCR reactions were carried out for 50 cycles (95°C for 35 sec., 62°C for 60 sec., 72°C for 60 sec.) in the iCycler iQ Real Time Detection System (Bio-Rad). 10 μ l of PCR cocktail included 1 μ l diluted archival tissue lysate, 1 unit heat-activated Taq DNA polymerase, 1 \times PCR buffer (pH 8.3), 2.5 mM MgCl₂, 200 μ M dNTP, 0.5 μ M each primer, and 0.5 \times SYBR Green I. The specificity of the 134-bp PCR product was confirmed by melting curve analysis. In order to control the accuracy of the real-time genotyping, a conventional gel-based allele-specific PCR (35 cycles in the same conditions) was applied to all mutation-positive samples as well as to some randomly selected mutation-negative DNA specimens.

Results

An example of the detection of BRCA1 4153delA mutation is presented in fig. 1. As seen in this figure, allele-specific PCR allowed reliable discrimination between mutated and wild-type alleles in both real-time and conventional format.

An analysis of 177 unselected ovarian cancer cases allowed only two 4153delA mutation carriers (1.1%) to be revealed. Both cases were early onset (39 and 42 years, respectively), and had identical morphological characteristics (T3NxM0, serous histology, grade 3). Records on family history in the present data set were incomplete and therefore not considered in the study analysis; nevertheless, one of the BRCA1 4153delA carriers reported an ovarian cancer in her grandmother.

Since the observed frequency of the 4153delA allele was lower than expected, part of the DNA samples was

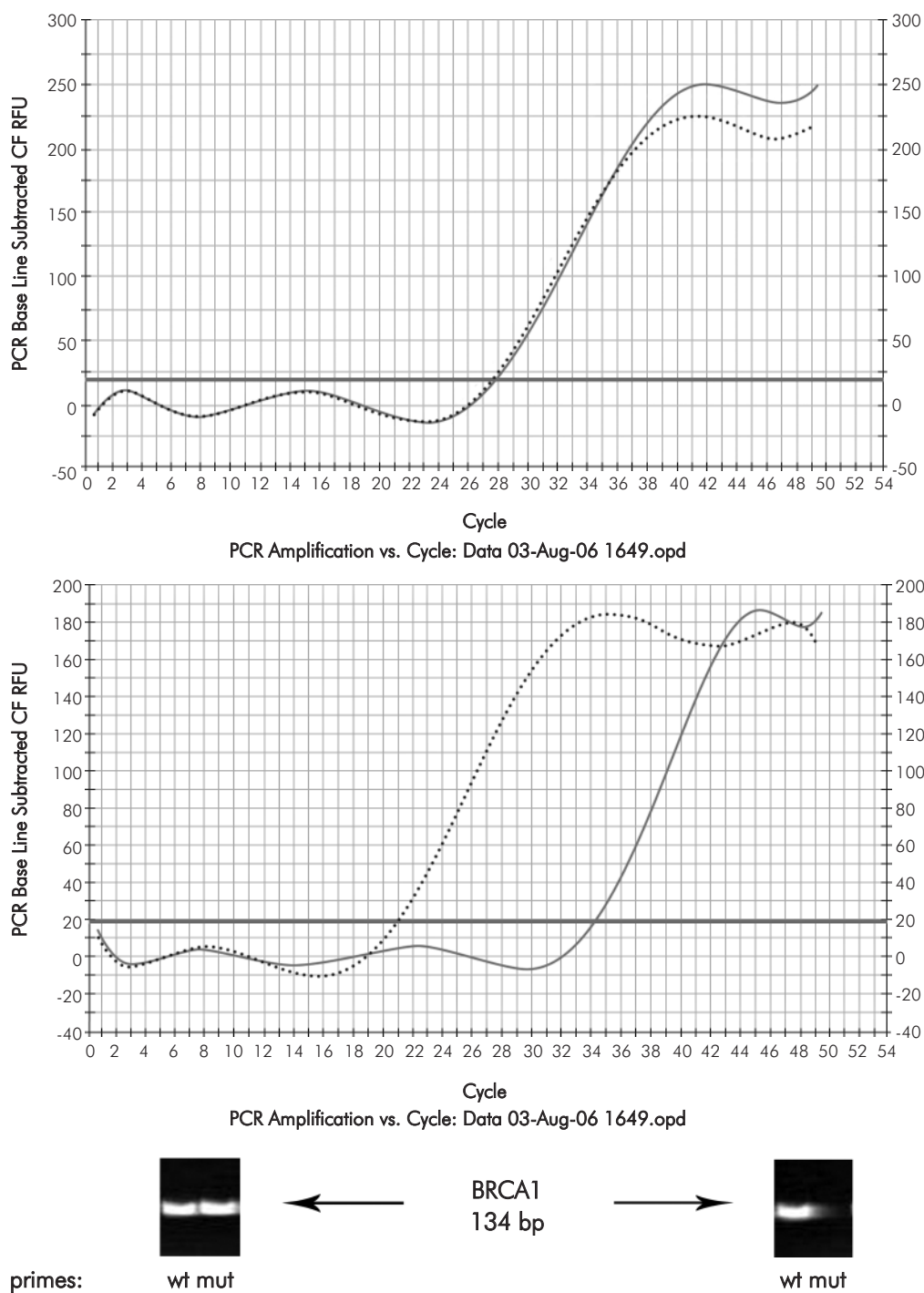


Fig. 1. BRCA1 4153delA genotyping. Both wild-type and mutation-specific primers produce the PCR fragment for the heterozygote (left), while only one product is detected upon the analysis of normal DNA sample (right). Identical results are obtained by real-time (top) and conventional (bottom) allele-specific PCR

genotyped for BRCA1 5382insC mutation. BRCA1 5382insC occurs in up to 10% of familial and/or early-onset and/or bilateral breast cancer patients in Russia [16]; therefore it is likely to be frequent in ovarian

cancers as well. An analysis of the 5382insC variant was performed as a positive control for 21 OC samples from our series, and 5 mutations (23.8%) were revealed. Therefore we conclude that the low frequency

of BRCA1 4153delA is not an artefact of the study design, but reflects the true contribution of this allele in Russian ovarian cancer.

Discussion

This study failed to reveal elevated incidence of BRCA1 4153delA mutation in unselected Russian patients suffering from ovarian cancer. BRCA1 4153delA was detected in several East European countries; however, its frequency significantly varies even within closely located geographic regions [8, 9, 11, 12]. The uneven distribution of 4153delA allele carriers may reflect a relatively recent origin of this genetic variant.

The data obtained in the present study are strikingly similar to the results of the analysis of Russian familial and/or early-onset and/or bilateral breast cancer [Sokolenko et al., manuscript submitted]. In particular, we observed moderate frequency of BRCA1 4153delA mutation (2/177 (1.1%) for OC and 3/302 (1.0%) for BC), but highly elevated occurrence of the BRCA1 5382insC variant (5/21 (23.8%) for OC and 29/302 (9.6%) for BC). Nonetheless, given the presumably high penetrance of BRCA1 truncating mutations, even the frequency of 1% in cancer patients seems to be sufficient justification for extended genetic testing. Therefore, we believe that BRCA1 4153delA mutation has to be included in the array of tests aimed at revealing cancer syndrome carriers among subjects of Russian origin.

In conclusion, this study supports neither the Russian origin of BRCA1 4153delA mutation [5, 6] nor its selectivity towards ovarian versus breast cancer predisposition [4, 11, 14]. However, the 4153delA variant does make a moderate contribution to breast and ovarian cancer incidence in Russia; thus it has to be considered in clinical practice.

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