
HUMAN
GENETICS

Prevalence of Widespread *BRCA1* Gene Mutations in Patients with Familial Breast Cancer from St. Petersburg

N. A. Grudinina¹, V. I. Golubkov¹, O. S. Tikhomirova¹, T. V. Brezhneva¹,
K. P. Hanson², V. B. Vasilyev¹, and M. Yu. Mandelshtam¹

¹ *Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg, 197376 Russia; fax: (812) 234-94-89; e-mail: michail@MM13666.spb.edu*

² *Petrov Research Institute of Oncology, Ministry of Health of the Russian Federation, pos. Pesochnyi-2, St. Petersburg, 189646 Russia; fax: (812) 596-89-47; e-mail: root@irion.spb.ru*

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Abstract—Ten variants different from the canonical nucleotide sequence (GenBank, U14680) has been identified when studying the mutation spectrum in gene *BRCA1*. Six of them (*5382insC*, *2963del10*, *3819del5*, *3875del4*, *2274insA*, and *R1203X*) cause premature termination of protein synthesis, thus predisposing to breast cancer. A missense mutation *E1250K* is presumed to be a factor of predisposition to cancer. We classified three variants of nucleotide sequence found in a number patients as DNA polymorphisms *S694S*, *L771L*, and *E1038G*. The *5382insC* and *3819del5* mutations have been recorded in four and two families, respectively. Five of the mutations detected have not been found in Russia before. However, all mutations except for *2963del10* have been found in other populations of the world, which indicates their long evolutionary history. Two mutations found in patients from St. Petersburg (*5382insC* and *3875del4*) have also been found in oncological patients from other regions of the Russian Federation.

INTRODUCTION

About 10% of breast cancer cases are related to inherited mutations *BRCA1* and *BRCA2*. In families with hereditary predisposition caused by these mutations, early-onset breast or ovarian tumors are usually found in several relatives, with breast cancer sometimes being bilateral [1]. Information on the regional mutation spectra in genes *BRCA1* and *BRCA2* allows rapid diagnosis of these genetic defects and determination of cancer risk groups in populations. In addition, the identification of mutations in the *BRCA* gene family makes it possible to find functionally regions of important protein encoded by these genes. In Russia, studies on *BRCA* gene mutations began relatively recently [2–6]; however, even the few available data show that the *5382insC* mutation of the *BRCA1* gene is the most prevalent in Russia. Many patients with familial breast cancer are not carriers of the *5382insC* mutation; therefore, other mutations in this gene should be searched for. After *5382insC* and other mutations of exon 20 were excluded in our patients [4], we scanned exon 11 (the longest exon of the *BRCA1* gene) in the families of 43 patients with breast cancer. Here, we present the results of this study.

MATERIALS AND METHODS

Patients. Medical geneticists from the Petrov Research Institute of Oncology of the Ministry of Health of the Russian Federation selected patients with breast cancer on the basis of the diagnostic signs of its

familial form, namely, the early onset and/or the presence of at least three cases of breast/ovarian cancer in the patient's pedigree. After the probands gave informed written consent for testing, we collected more detailed family anamneses and took blood samples from probands. We also suggested that the relatives of the probands carrying the mutations studied should be tested.

Biochemical methods. To isolate DNA, 5-ml samples of venous blood were taken from the patients. The blood was sampled in test tubes containing EDTA solution and stored at -20°C . The DNA was isolated using the standard Kunkel's [7] method modified by Bell *et al.* [8]. For amplifying individual *BRCA1* exons, we used oligonucleotide primers synthesized in Litekh (Russia) on the basis of published sequences [9]. Exon 11 of the *BRCA1* gene is very large; therefore, it was subdivided into several overlapping segments denoted 11A–11P [9], which were analyzed separately.

Polymerase chain reaction (PCR) was performed in a Tertsik thermal cycler (Russia). Reagents from Medigen (Russia) were used. Mixtures for PCR were 30 μl in volume and contained 0.25 μM of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.4 at room temperature), 1.5 mM MgCl_2 , 200 μM of each deoxynucleotide triphosphate, 1 U of *Taq* polymerase, and 20–100 ng of genomic DNA. The durations of the steps (the stages of DNA denaturing, primer annealing, and the synthesis of new DNA strands) in each cycle of PCR were 1 min. Only the annealing temperature was varied for different pairs of primers.

The products of PCR were identified by electrophoresis in 8% polyacrylamide gel buffered with single Tris-borate followed by silver staining for DNA. For a better identification of heteroduplexes and single-stranded DNA conformers during single-strand conformation polymorphism (SSCP) analysis, we used Tris-glycine buffer and polyacrylamide gels at different concentrations.

We used a linearized plasmid from the TAKLON kit (Medigen, Russia) and a ligase obtained from Amersham Biosciences (United Kingdom) for direct cloning of the amplification products. We transformed *Escherichia coli* strain DH5 α with plasmid containing cloned sequences of the *BRCA1* gene by the calcium chloride method and isolated the plasmids for PCR or sequencing by the alkaline lysis method [10]. Recombinants were selected on a medium containing an antibiotic and the X-Gal chromogen [10]. For DNA sequencing, we used an ALF Express-2 automatic sequencer (Amersham Biosciences) with the use of fluorophore-labeled Cy-5 primers or dideoxynucleotide triphosphates. In some cases, we used an ABI 377 device (Applied Biosystems) for sequencing.

The control DNA sample from a heterozygous carrier of the *5382insC* was obtained from E. Levy-Lahad (Israel).

RESULTS

In DNA samples from 43 patients with a well-documented family history of breast cancer, we found four cases of the *5382insC* mutation in the *BRCA1* gene [4]. Afterwards, we analyzed exon 11 of the *BRCA1* gene in all patients to find the possible variants of the nucleotide sequence. We searched for mutations in each of the 11A–11P fragments with the use of SSCP analysis. Long-path electrophoresis effectively identified heteroduplexes under these conditions (Fig. 1).

Screening of the 11J fragment detected a sample with characteristic heteroduplexes. The DNA electrophoresis under denaturing conditions yielded two discrete zones. An additional zone of DNA with an electrophoretic mobility higher than in normal DNA samples indicated the presence of a large deletion. After cloning and sequencing the mutant allele (Fig. 2), we found the absence of ten nucleotides in it. According to the existing nomenclature, this mutation was denoted *2963del10*. Family analysis (Fig. 3) demonstrated that the proband's sister and daughter carried this mutation.

The analysis of SSCP fragment 11O yielded several samples with additional single- and double-stranded fragments (Fig. 1). Cloning and sequencing DNA from two unrelated patients showed a similar five-nucleotide deletion *3819del5* and, in one patient, a four-nucleotide deletion *3875del4*. Direct sequencing of the amplified fragments made it possible to detect a nonsense mutation *R1203X* in the same exon of one proband and a missense mutation *E1250K* in that of the other proband.

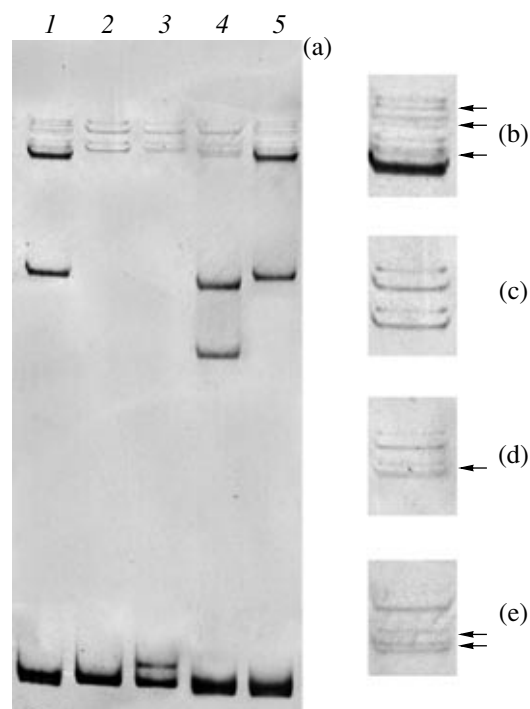


Fig. 1. Identification of mutations in exon 11O of the *BRCA1* gene with the use of SSCP analysis. Single-stranded DNA conformers were separated from homo- and heteroduplexes by electrophoresis in 12% polyacrylamide gel at room temperature. DNA was silver stained. Lanes: 1, 5, heterozygous carriers of the *3819del5* mutation (inset b); 2, a sample with a normal nucleotide sequence (inset c); 3, a heterozygous carrier of the *E1250K* mutation (inset d); and 4, a heterozygous carrier of the *3875del4* mutation (inset e). (a) Intensely stained homoduplex zones (bottom), heteroduplex zones, and less intensely stained single-stranded conformer zones (top) are seen. Differences in the distribution pattern of single-stranded conformers for individual mutations are shown in insets (b–e) on the right of (a). Arrows on the right of the insets show additional zones of single-stranded DNA conformers as compared to the normal nucleotide sequence.

A one-nucleotide insertion *2274insA* was found in fragment 11G in one case. The screening of the remaining fragments of exon 11 detected only widespread variants of the nucleotide sequence of the *BRCA1* gene known as DNA polymorphisms. Table 1 shows a complete list of mutations and polymorphisms found in this study.

DISCUSSION

About 1200 mutations and polymorphisms are known in the *BRCA1* gene [11]. The mutation spectra vary considerably in different populations. Information on the *BRCA1* gene mutation spectrum permits the development of the methods for rapidly testing the mutations that are prevalent in each particular population, thus substantially accelerating the identification of genetic defects in the relatives of patients with breast cancer. Data on the spectrum of *BRCA1* gene mutations in Russia are insufficient [2–6], which determined the

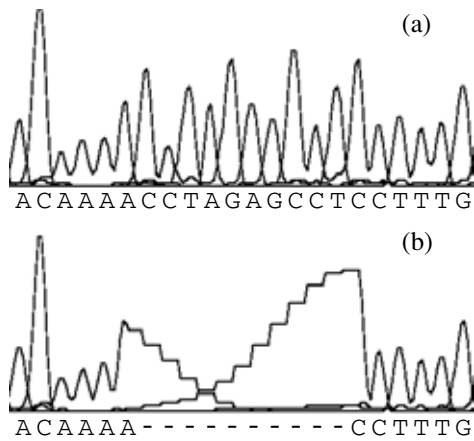


Fig. 2. Identification of the 2963del110nt mutation in exon 11 of the *BRCA1* by means of automated DNA sequencing. (b) The mutant allele lacks ten nucleotides compared to (a) the normal allele (shown by dashes).

subject of our study. In 43 patients from St. Petersburg, we found seven different mutations and three polymorphisms in exons 11 and 20 of the *BRCA1* gene (Table 1).

In four probands, we found the 5382insC mutation, which shifted the reading frame and formed a stop codon at position 1829. The amino acid sequence of the protein changed beginning from amino acid residue 1756, which resulted in a partial loss of BRCT repeats between amino acid residues 1640 and 1863. Their loss may disturb DNA repair and cell-cycle regulation, thus leading to cancer. Epidemiological data confirm the role of 5382insC in the development of breast/ovarian cancer [1, 11]. The 5382insC mutation was absent in probands' daughters in two out of four affected families.

The formation of heteroduplexes in heterozygous carriers of the 5382insC mutation allows its rapid detection. Heteroduplex analysis can be used as the primary method when searching for the 5382insC mutation in pedigrees with familial breast cancer. The 5382insC mutation is very frequent in Europe, including Poland [12, 13] and Czech Republic [14], probably, because of its Eastern European origin [15].

Mutation 3819del5 was found in two related patients with breast cancer first diagnosed at ages of 31 and 36 years, respectively. In the latter patient, primary cancer of the other mammary gland was diagnosed at an age of 48 years. This mutation causes a reading-frame shift, which results in the formation of a stop codon at position 1262. This mutation has been found in several European countries, including Germany, Denmark, Czech Republic, France, and Poland, as well as in Australia and the United States [11].

Mutation 3875del4 located in the same fragment of exon 11 also results in a reading-frame shift and the formation of a stop codon (at position 1241). We found it in a patient with breast cancer diagnosed at an age of 47 years and in her daughter who had had lobular hyper-

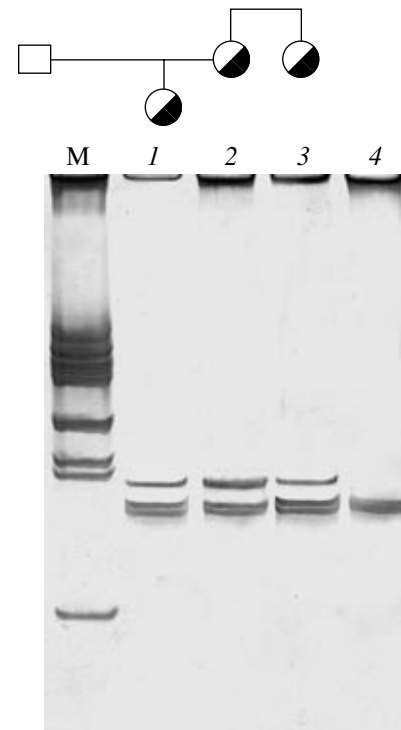


Fig. 3. The inheritance of the 2963del110 mutation in the family of patient no. 2041. The proband's pedigree is shown above the electrophoregram. The products of the amplification of exon 11J fragments are shown under the symbols of pedigree members. Lanes: M, a marker of molecular weights from 100 to 1000 bp at a step of 100 bp; 1–3, the products of the amplification of fragment 11J of the *BRCA1* gene in a family with the 2963del110 mutation; 4, the product of the amplification of the same gene fragment from a patient without this mutation. Additional bands formed by heteroduplexes are seen in DNA samples from carriers of the mutation. Breast cancer was diagnosed in the proband (the PCR product in lane 2) at an age of 41 years; ovarian cancer, in the proband's sister (lane 3) at an age of 52 years; the proband's daughter (lane 1) had no signs of cancer at an age of 19 years.

plasia of the mammary gland and right ovarian cyst at an age of 14 years. The 3875del4 mutation has been found in most populations of Europe (Italy, Ireland, Scotland, England, Denmark, Czech Republic, and Germany), as well as in Asia and Africa [11, 16]. This is the fourth most frequent mutation among those resulting in a reading-frame shift [11]. The 3819del5 and 3875del4 mutations accelerate mRNA degradation [17].

Mutation 2274insA, which we found in a patient with familial breast cancer, also results in a reading-frame shift and may cause malignity.

This also applies to the novel mutation 2963del110. It, as well as 14 other mutations of the *BRCA1* gene known to date, results in a stop codon (at position 999). These data and family analysis lead to the conclusion that the 2963del110 mutation also predisposes to breast cancer.

Mutation *R1203X* was found in a patient with familial breast cancer. It leads to the synthesis of a truncated protein and substantial disturbances in the repair of double-strand DNA breaks [18]. The *R1203X* mutation has been found in most populations of Western Europe [11]. Family analysis has demonstrated that a proband's relative also carried an *R1203X* mutation.

In addition to the reading-frame-shift mutations and the nonsense mutation, we found one missense mutation, *E1250K*. There are no data on its role in cancer [11]. In our study, however, the daughter of the proband that carried the same mutation developed breast cancer at an age of 39 years, which suggests that the *E1250K* mutation plays a role in the pathogenesis of cancer.

Some *BRCA1* polymorphisms (*S694S*, *L771L*, and *E1038G*) that were earlier described in many populations of the world have been found in St. Petersburg (Table 1). The high frequencies of all these alleles in the sample studied, the presence of two types of homozygotes for each polymorphism, and the compliance with the Hardy-Weinberg equilibrium with respect to genotype frequencies in the sample of patients from St. Petersburg are indirect evidence that these polymorphisms do not contribute to cancer. The genotyping of the patients demonstrated that the alleles of the three polymorphisms were linked with one another (Table 2), which was earlier found in the Canadian population [19] and in patients from Moscow [6].

Thus, our analysis of exons 11 and 20 of the *BRCA1* gene in 43 patients with familial breast cancer has revealed ten variants differing from the canonical nucleotide sequence (GenBank U14680). Seven of these variants (*5382insC*, *2963del10*, *3819del5*, *3875del4*, *2274insA*, *R1203X*, and *E1250K*) have been characterized as alleles predisposing to breast cancer. Three more variants are polymorphisms described earlier. Five mutations have been found in Russia for the first time, and one of them (*2963del10*) has not been described before at all. Two mutations detected in patients from St. Petersburg (*5382insC* and *3875del4*) were earlier found in other regions of Russia [3, 6]. The finding of frequent mutations of the *BRCA1* gene in St. Petersburg, as well as in many other populations, indicate a considerable evolutionary age of these DNA variants. Apparently, an immediate goal is to find in oncological patients from St. Petersburg other mutations that are prevalent in Europe and the white population of the United States. This would help to identify the mutations even before the entire gene has been sequenced. The latter approach is preferred, e.g., in the case of familial hypercholesterolemia, where most mutations are specific for each population or ethnic group. Conversely, in the case of inherited mutations predisposing to breast cancer, it may be possible to construct DNA chips for diagnosing Russian mutations of the *BRCA1* gene on the basis of the mutations most prevalent in Europe.

Table 1. Characteristics of mutations in *BRCA1* gene exons identified in breast cancer patients from St. Petersburg

Exon	Mutation	Effect	Number of families (patients)
20	<i>5382insC</i>	Reading-frame shift	4 (4)
11J	<i>2963del10</i> **	The same	1 (3)
11O	<i>3819del5</i>	"	2 (2)
11O	<i>3875del4</i>	"	1 (2)
11G	<i>2274insA</i>	"	1 (1)
11O	<i>R1203X</i> *	CGA > TGA c.3726 C > T	1 (2)
11O	<i>E1250K</i>	GAG > AAG c.3867 G > A	1 (2)
11H	<i>L771L</i> *	TTG > CTG c.2430 T > C	Frequent polymorphism
11K2	<i>E1038G</i> *	GAA > GGA c.3232 A > G	Frequent polymorphism
11G	<i>S694S</i> *	AGC > AGT c.2201C > T	Frequent polymorphism

* Mutations identified only by SSCP analysis. The other mutations are also easily identified by the formation of characteristic heteroduplexes.

** A novel mutation; the remaining variants of the nucleotide sequences of this gene have been known before.

Table 2. The linkage of the alleles of three polymorphisms in the *BRCA1* gene

Polymorphism	Allele	
<i>S694S</i> (c.2201 C > T)	C	T
<i>L771L</i> (c.2430 T > C)	T	C
<i>E1038G</i> (c.3232 A > G)	A	G

Note: Nucleotides are enumerated according to the *BRCA1* cDNA nucleotide sequence, which is indicated by letter c (from cDNA).

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