

## Analysis of Several BRCA1 and BRCA2 Mutations in a Hospital-based Series of Unselected Breast Cancer Cases

T. Burcoş<sup>1</sup>, D. Cimponeriu<sup>2</sup>, D.A. Ion<sup>3</sup>, S. Spandole<sup>2</sup>, P. Apostol<sup>2</sup>, M. Toma<sup>2</sup>, I. Radu<sup>2</sup>, I. Popa<sup>1</sup>, S. Stanilescu<sup>1</sup>, E. Popa<sup>1</sup>

<sup>1</sup>Department of Surgery, Coltea Hospital, Bucharest, Romania

<sup>2</sup>University of Bucharest, Department of Genetics, Bucharest, Romania

<sup>3</sup>"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

### Rezumat

#### *Analiza unor mutații în genele BRCA1 și BRCA2 într-o serie de cazuri de cancer de sân selectate dintr-un singur spital*

Distribuția mutațiilor în genele BRCA variază în mod semnificativ între populații. Spectrul mutațional al genelor BRCA1 și BRCA 2 în cazurile de cancer de sân în populația din România este parțial cunoscut. Scopul acestui studiu a fost de a investiga prezența a nouă mutații BRCA la pacienți cu cancer de sân identificate într-o clinică chirurgicală din București.

**Metodă:** Pentru acest studiu au fost selectate femei diagnosticate cu cancer de sân la Spitalul Colțea (n = 114) și femei sănătoase (n=150). Șapte mutații în BRCA1 (185delAG, 5382insC, 943ins10, E1250X, 1294del40, E1373X, R1443X) și două în BRCA2 (IVS16-2A4G și 6174delT) au fost testate folosind protocoale PCR. În plus, prezența mutațiilor BRCA1 185delAG, BRCA1 5382insC și BRCA2 6174delT a fost testată cu un sistem de detecție a mutațiilor post-amplificare, bazat pe metoda ELISA.

**Rezultate:** Două pacienți cu cancer de sân sporadic (2%) și o pacientă cu istoric familial de boală (7,14%) au prezentat mutația BRCA1 5382insC. Nicio altă mutație nu a fost detectată la pacienți sau în lotul control. În lotul control nu au fost identificate mutații.

**Concluzii:** Rezultatele noastre indică faptul că BRCA1 5382insC este o mutație comună la femeile cu cancer de sân din România (3/114).

**Cuvinte cheie:** cancer de sân, mutații BRCA, BRCA1 5382insC, teste genetice

### Abstract

**Background:** The distribution of BRCA mutations varies significantly between populations. The spectrum of BRCA1 and BRCA2 mutations in breast cancers in the Romanian population is incompletely known. The aim of the present study is to investigate the presence of nine BRCA mutations in patients with breast cancer identified in a surgical clinic from Bucharest.

**Methods:** Unrelated women diagnosed with breast cancer from Coltea Hospital (n=114) and healthy controls (n = 150) were selected for this study. Seven mutations in BRCA1 (185delAG, 5382insC, 943ins10, E1250X, 1294del40, E1373X, R1443X) and two in BRCA2 (IVS16-2A4G and 6174delT) were tested using PCR based protocols. In addition, the presence of BRCA1 185delAG, BRCA1 5382insC, BRCA2 6174delT mutations were tested with a post amplification mutation detection system, based on the ELISA method.

**Results:** Two patients with sporadic breast cancer (2%) and one patient with family history of the disease (7.14%) have the BRCA1 5382insC mutation. No other mutation was detected in patient and control groups. The mutations were not present in the control lot.

**Conclusions:** Our results indicate that BRCA1 5382insC is a common mutation in Romanian women with breast cancer (3/114).

Corresponding author:

Emil Popa, MD, PhD  
Department of Surgery, Coltea Hospital  
I.C. Bratianu street, no 1, 3th district  
Bucharest, Romania  
E-mail: emilpopa@yahoo.com

**Key words:** breast cancer, BRCA mutations, BRCA1 5382insC, genetic test

## Introduction

BRCA1 and BRCA2, similar to classic tumor suppressor genes, frequently lose their wild-type allele in malign tissues of mutation carriers. Mutations in these genes were reported to increase the risk for several malignancies, including breast and ovarian cancers, papillary serous carcinoma of the peritoneum. It is estimated that women who inherit a mutated copy of either gene have a 45% to 65% risk of developing breast cancer by the age of 70 (1). Overall, mutations in BRCA1 and BRCA2 account for about 2% of all breast cancer types.

The distribution of BRCA mutations is considered to present significant differences between populations. One of the first reports showed that the BRCA1 185delAG, BRCA1 5382insC and BRCA2 6174delT are common in Ashkenazi Jews. Lately, other mutations were found to be common in Polish (BRCA1 5382insC, C61G and 4153delA), Dutch (BRCA1 2804delAA), Chinese (BRCA1 1081delG), Russian (BRCA1 4153delA, 5382insC), and African-American (BRCA1 1832del5, 5296del4) populations. It is estimated that roughly 1/40-1/800 individuals carry one mutation that increases the risk for breast and/or ovarian cancer in general populations (2-4). The genetic structure and sub-structure of populations are influenced by different factors. Thus, significant differences in mutation distribution may be present between ethnical groups living in the same region (5,6).

Although the frequency of breast cancer in Romania has increased continuously during the last years, testing for BRCA mutations is not commonly available for physicians (7). Therefore, the spectrum of BRCA1 and BRCA2 mutations in sporadic and familiar breast cancers in our population is incompletely known.

The aim of the present study is to investigate the presence of nine BRCA mutations in patients with breast cancer identified in a surgical clinic from Bucharest.

## Material and Method

Unrelated consecutive women with breast cancer (100 women with sporadic breast cancer and 14 women with family history of disease) confirmed by pathology report, regardless of family history or age at onset from Coltea Hospital (Bucharest) were included in the analysis.

All patients diagnosed with breast cancer were clinically examined. Based on this examination pre-treatment staging was performed. All patients were also examined by mammography or ultrasound. In some difficult cases diagnostic MRI was needed. In all cases histological specimens were obtained, which is the examination that confirmed the diagnosis.

Histopathological diagnosis was made by fine needle aspiration, core needle biopsy or excision biopsy in a few cases. Some patients were examined histologically after

mammectomy or after conservative surgery for the disease, according to stage. In all cases the biopsy pieces were analysed by immunohistochemistry establishing the presence of estrogen and progesterone receptors and the degree of aggressiveness of the tumour (by determining c-erb and the proliferation marker Ki67) (12).

Breast cancer patients have undergone therapeutic protocols according to disease stage. This involved initial chemotherapy for cases with clinically positive lymph nodes or large tumours. Patients with positive estrogen or progesterone receptors received specific anti-hormonal treatment (aromatase inhibitors) or surgical ooforectomy.

Most patients underwent surgery, the overwhelming majority of cases practicing the modified mammectomy Madden. In a small percentage of cases conservative surgery for breast cancer could be performed (13). In these cases, the surgical sequence was followed by irradiation for proper consolidation of therapy.

All specimens obtained by mammectomy were analysed in terms of settling histopathological tumour grading, tumour size, lymphovascular, perineural and perimammary fatty tissue invasion. All nodes were examined to determine the extent of the disease, with a minimum of 12 lymph nodes examined before deciding on an N0 grading (14).

Healthy women (n=150) without known family history of malignancies were selected from subjects who attended different clinics in Bucharest for routine checking of their health status. These patients were examined clinically and by ultrasound or mammography, excluding the cases detected with breast lesions (malignant or benign).

Blood samples from all subjects and 30 fresh tumor specimens obtained during the surgical resection of breast cancer were collected. Tissues selected from the middle of tumor specimens were disrupted to the cellular tissue with a Potter-Elvehjem Tissue Grinders (Micro Tissue Grinder Kit, Cole - Parmer). DNA was extracted from these cellular tissues (30 mg) and from blood samples (300  $\mu$ l) using Wizard DNA Extraction Kit (Promega, SUA). Prior to being used for PCR, the DNA was quantified with Quant-iT™ PicoGreen® (Invitrogen Corp., Carlsbad CA) in a Rotor - Gene 6000 (Corbett Research) and was tested for integrity by agarose gel electrophoresis.

### *Mutation detection*

Seven mutations in BRCA1 (185delAG, 5382insC, 943ins10, E1250X, 1294del40, E1373X, R1443X) and two in BRCA2 (IVS16-2A4G and 6174delT) were tested using the PCR based protocols. Primer sequences and PCR conditions were similar to those described previously (8-11). Briefly, the PCR (Corbett Research thermocycler) protocol consists in an initial denaturation step at 94°C for 2 min, followed by 30 cycles of 94°C for 1 min, annealing at specific temperature for 40-60 sec, extension at 72° for 1 min, and a final extension step at 72° for 2 min. Each PCR mixture (15  $\mu$ l) contains genomic DNA (50 ng), primers (each 50 ng), Taq pol (1.5 U), dNTP (0.2 mM).

The genotypes for BRCA 943ins10 and 1294del40 were

established after electrophoresis of amplicons in neutral polyacrylamide gel (8%, TBE 1x, 5V/cm). A duplex PCR was used for detection of E1373X mutation. The normal allele does not yield any product, whereas amplicons with mutation have 120bp. The second primer pairs represent an internal control the amplification reaction. PCR products were separated on agarose gel (2%, TBE 1x, 5V/cm). The other amplicons require restriction with DdeI, StyI, AlfIII or BstNI endonucleases before PAGE (8%).

The presence of BRCA1 185delAG, BRCA1 5382insC, BRCA2 6174delT mutations were tested in all samples with a post amplification mutation detection system, based on the ELISA method (PRONTO® BRCA kit, Pronto Diagnostic, Israel). We performed a visual detection of mutations. All samples were interpreted independently by two researchers, to ensure a correct genotyping procedure.

The amplicons were also analysed by the single-strand conformation polymorphism (SSCP) method. Briefly, 2  $\mu$ l of amplicon were mixed with 10  $\mu$ l loading buffer, denatured at 95°C for 10 min, cooled rapidly on ice and then were resolved on a non-denaturing polyacrylamide gel for 6–10 h. Bands from the gels were revealed by silver staining.

#### Statistical analysis

The distribution of genotypes in accordance with Hardy-Weinberg equilibrium was tested using  $\chi^2$  test. The difference in genotype distribution between patient and control lots was analysed with Fisher exact test (15).

The subjects self-reported to be Romanian Caucasian and provided a written informed consent before selection for this study. Ethical approval was obtained from the research committees of the Coltea Hospital.

## Results

In this study, we obtained DNA from biological samples collected from women with sporadic (n=100) or familial (n=14) breast cancer selected in a consecutive manner and from healthy control women (n=150). The average age of

patients ( $69.99 \pm 13.34$  years, range: 30-85 years) and healthy controls was similar ( $69 \pm 13.34$  years, range: 30-83 years). The DNA of Torque Teno virus was detected by PCR in 87% of patients and in 62% of controls.

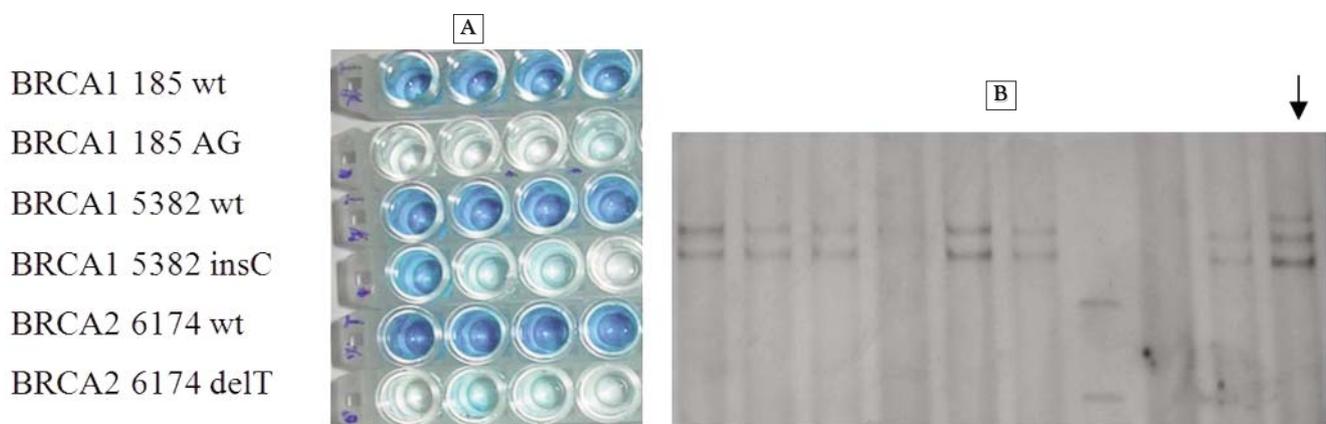
All DNA samples were successfully genotyped for nine mutations. We identified only the BRCA1 5382insC mutations in three patients from our lots. This mutation was detected using PCR-based methods (commercial kit "Pronto BRCA Kit" and classical protocols) in blood and tumor samples from each of three patients (Fig. 1). BRCA1 5382insC mutation was distributed according to Hardy-Weinberg equilibrium and the difference in distribution between patient and control lots was not statistically significant ( $p > 0.05$ ).

## Discussions

A high number of pathogenic mutations and polymorphisms with unknown significance were detected in BRCA1 and BRCA2 genes (16). Mutations related to breast cancer are several times more frequent in hereditary form of cancer than in the sporadic disease. Aggregation of breast cancer within the family is the most important factor that increases the probability to be a carrier of BRCA mutations. Different guides were published to increase the cost-efficiency of genetic tests for BRCA mutations (17-23). However, increasing the stringency of selection criteria reduces the chance to identify new BRCA mutations, especially in sporadic forms of cancers.

Screening for common mutations in BRCA1 and BRCA2 has become a routine part of the investigation and management of familial breast and ovarian cancer. There are limited data regarding BRCA gene mutations or polymorphisms in our population because these genetic tests are not currently provided by Romanian hospitals (24,25).

In this study we have tested the presence of nine mutations in consecutive women with breast cancer presented in a single surgical clinic from Bucharest and in control subjects. Among these, only 5382insC mutation in exon 20 of BRCA1 was found in three patients with breast cancer. The classical protocol, the detection kit and SSCP confirm the presence of



**Figure 1.** Analysis of three mutations in BRCA genes using BRCA Pronto Kit (A) and confirmation of BRCA1 5382 insC by SSCP analysis (B)

this mutation in the blood and tumor tissue in heterozygous status. Thus, the BRCA1 5382insC germinal mutation has a high frequency in patients with breast cancer. This mutation determines a truncated BRCA1 protein that is believed to be more stable than the wild-type protein (26).

The BRCA1 5382insC was not present in our control lot. This result is in concordance with previous reports which estimated that the frequency of BRCA1 5382insC in the general population is under 0.5% (4,27,28).

The frequency of BRCA1 5382insC is much higher in breast cancer patients. Two patients from our lot of sporadic breast cancer have this mutation. Thus, the frequency of this mutation was higher than in unselected breast cancer patients of Ashkenazi Jewish (0.75%) (24), German (1.0%) (29) and Hungarian (1.4%) (28) origins.

The distribution of BRCA1 5382insC in breast and ovarian cancer high-risk families presents significant differences between populations. Thus, it was not identified in families with breast cancer from Sweden (30,31), Norway (32) and Chile (33) or it was found to be relatively common in families from Germany (29), Canada (34), Hungary (35) and Romania (25). BRCA1 5382insC is well represented compared with other BRCA mutations in families with breast/ovarian cancer patients from Poland (55%) (36). We found this mutation in 1 of 14 cases with family history of breast cancer, similar with data previously presented.

Based on the analysis of BRCA1 5382insC frequency and its geographical distribution (34,35,37,38) this mutation was considered to have originated in the Baltic area during the medieval period and it has been spread through migration. This may explain its prevalence in Central and Eastern Europe and its decreasing prevalence from the East to the West regions of Europe (39-43).

## Conclusion

Our results indicate that BRCA1 5382insC is a common mutation in women with breast cancer from the Romanian population.

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