

## Prevalence of *BRCA1* and *BRCA2* Mutations in Korean Breast Cancer Patients

The incidence of breast cancer in Korea has been increasing in recent years, such that it is now the most common female cancer. Breast cancer in Korea is characterized by an earlier age of onset than in Western countries, suggesting that it would be related with genetic background. We assayed germline mutations in the *BRCA* genes to evaluate their genetic pathology in Korean breast cancer patients. The study subjects consisted of 173 patients at clinically higher risk and 109 unselected patients. Germline mutations in the entire coding sequences of the *BRCA1* and *BRCA2* genes were analyzed by Conformation-Sensitive Gel Electrophoresis (CSGE), and any aberrantly-sized band was sequenced. *BRCA* mutations were present in 12.7% of the high risk patients, compared with 2.8% of the unselected patients. Among high risk patients, mutations were most prevalent in patients with a family history of breast or first-degree ovarian cancer (22.1%), followed by those with male breast cancer (20%), bilateral breast cancer (20%), multiple organ cancer including breast (13%) and younger breast cancer patients (aged <35 yr) (8.1%). Moreover, *BRCA* mutations were detected in 34.8% of patients having two high-risk factors. These findings suggest that *BRCA* gene mutation analysis should be performed on Korean patients with high-risk factors for breast cancer.

Key Words : Breast Neoplasms; Genes, *BRCA1*; Prevalence; Koreans

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## INTRODUCTION

The incidence of breast cancer in Korea has been increasing in recent years. Although this cancer is still much less prevalent in Korea than in Europe and the United States, it has been the most common cancer in Korean women since 2001 (1). Remarkably, the peak age of Korean women presenting with breast cancer is in the 40's, a peak age 15-20 yr younger than in the United States. One of the characteristics of hereditary breast cancer is a tendency towards younger age at onset. Breast cancer is typically diagnosed at a young age in Southeast Asia, including Korea, suggesting that genetic factors may be important (2).

Although 20% to 25% of breast cancers are familial (i.e., associated with a family history of breast cancer), in Western countries only 5% to 10% are hereditary, with an autosomal dominant genetic determinant. Most hereditary breast cancers are associated with mutations in the *BRCA1* (MIN 113705) and *BRCA2* (MIN 600185) genes (3), which confer high susceptibility to familial breast and/or ovarian cancer (4, 5). Ethnicity plays a role in hereditary breast cancer through its asso-

ciation with particular "founder" mutations. For example, founder mutations have been identified in various ethnic groups, including Icelanders, Ashkenazi Jews, Russians, and Israelis. In Iceland, a single *BRCA2* mutation (*BRCA2* 999del5) is found in 24% of women diagnosed with breast cancer below age 40 yr (6, 7). Twelve percent of unselected Jewish breast cancer cases, including 28% of those diagnosed below age 50 yr, were found to carry 1 of 3 founder *BRCA* mutations (8).

In general, there is little data on the contribution of germline *BRCA* mutations to breast cancer in Asia. Among unselected breast cancer cases in the Philippines, the prevalence of *BRCA* mutations was found to be 5.1% (2). Although nine germ-line mutations were identified in 21 Korean families, including two or more affected first- or second-degree relatives with breast and/or ovarian cancer (9), knowledge of *BRCA* mutations in the overall Korean breast cancer population is scanty. To determine the prevalence of *BRCA* germline mutations in Korean breast cancer patients, we performed mutational analysis of the *BRCA1* and *BRCA2* genes in high-risk breast cancer patients and in unselected patients using Conformation-Sensitive Gel Electrophoresis (CSGE).

## MATERIALS AND METHODS

### Subjects

The study subjects included 173 breast cancer patients at higher risk of mutating *BRCA* genes at Asan Medical Center, Seoul, between March 2002 and February 2003 and 109 unselected breast cancer patients treated at Korea University Guro Hospital, Seoul, between January 2000 and December 2000. The high-risk patients included 86 with familial breast or first-degree ovarian cancer histories, 74 with a younger age at onset (<35 yr), 15 bilateral cancers, 5 male breast cancers, and 16 multi-organ cancers (five thyroid cancers, three cervical cancers, three stomach cancers, one ovarian cancer, one skin cancer, and three unknown cancers), including breast cancer. Of those with familial histories, seventy-one patients had a history of breast cancer (50 in first-degree relatives including 3 with a first-degree family history of ovarian cancer, 13 in second-degree relatives, and 8 in both first and second-degree relatives including 1 with a first-degree family history of ovarian cancer). Only 7 patients had first-degree ovarian cancer histories, and the information on the rest 8 patients was not available. The unselected patients were included randomly without the information about their family histories or other high-risk factors. Subjects were asked to fill out questionnaires to evaluate their personal and family histories, and blood specimens were collected for determination of *BRCA* mutations. Informed consent was obtained from all the subjects in this study.

### DNA purification from EDTA-blood

Genomic DNA was isolated from EDTA-blood using the Accuprep DNA Purification kit (Bioneer, Korea).

### Generation of polymerase chain reaction (PCR) products

*BRCA1* and *BRCA2* mutations were scanned as described previously (12), with modifications. PCR products were synthesized by amplification of 75 fragments overlapping the 22

coding exons of *BRCA1* and the 26 coding exons of *BRCA2*. PCR primers were designed to generate fragments of 220-503 bp in length covering all coding exons and at least 40 bp of each exon-intron flanking region. In order to produce 75 fragments for both genes, 31 *BRCA1* fragments were generated in 9 triplexes and 2 duplexes, and 44 *BRCA2* fragments were generated in 11 triplexes, 4 duplexes, and 3 singles. For generation of fluorescent PCR products, each forward primer was labeled at the 5' end with one of the phosphoramidite dyes, HEX (yellow), TET (green), or 6-FAM (blue), while the reverse primers were unlabeled. Primer sequences and multiplex PCR conditions are available on request (e-mail: dabb@labgenomics.com). Typically, PCR reactions were performed in a reaction volume of 15  $\mu$ L containing 1  $\mu$ L of DNA solution, 2.5 mM MgCl<sub>2</sub>, 1.25 mM dNTP, 0.5  $\mu$ M of each primer and 1 U of TaKaRa *Taq* polymerase (TaKaRa Biomedicals, Japan). The amplification protocol consisted of denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 57°C for 1 min, and extension at 72°C for 45 sec. To generate heteroduplexes, the samples were denatured at 95°C for 5 min, annealed at 68°C for 30 min and cooled at 4°C. A 0.3-1.0  $\mu$ L aliquot of each PCR product was subsequently used for F-CSGE analysis.

### Fluorescent conformation-sensitive gel electrophoresis (F-CSGE)

Samples were electrophoresed in 0.2 mm thick 12% polyacrylamide gels, containing a 99:1 ratio of acrylamide (BioRad, CA, U.S.A.) to 1,2 bis-(acrylyl)piperazine (Fluka, Switzerland) and 15% (v/v) formamide, in 1  $\times$  TBE buffer with a 36-well comb. Samples were separated on a standard ABI 377 sequencer using 1  $\times$  TBE. Each sample loading mixture contained 0.3-1.0  $\mu$ L of multiplexed PCR product, 0.2  $\mu$ L size standard labeled with TAMRA (GS500, Applied Biosystems, CA, U.S.A.), 0.5  $\mu$ L dextran blue (50 mg/mL), and 0.5  $\mu$ L freshly deionized formamide. 1-1.8  $\mu$ L aliquots of the samples were applied per lane. Electrophoresis was performed at 2,000 V for 4.5 hr at 42°C in 1  $\times$  TBE, and the data were analyzed

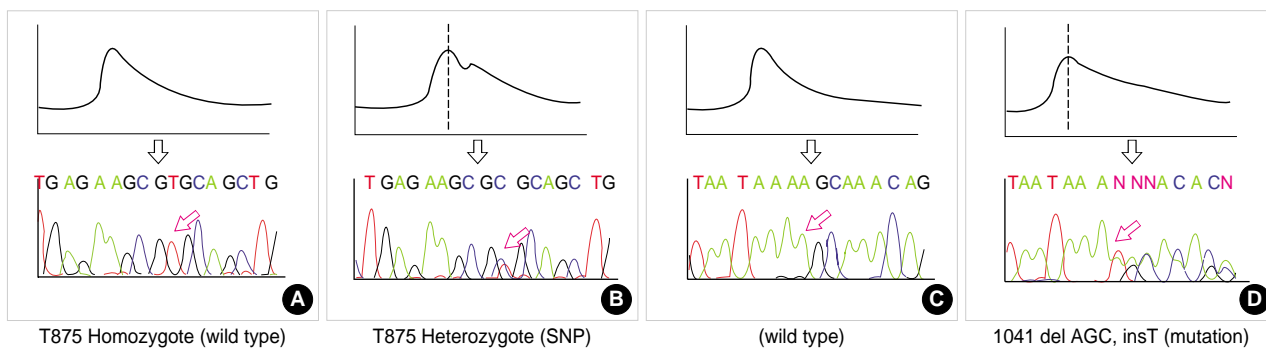


Fig. 1. A typical scan of a F-CSGE fragment which harbors the *BRCA1* sequence variations T875C (panel B) and 1041\_1043delAGCinsT (panel D) in comparison with the wild-type control (panel A, C).

with GeneScan software (Applied Biosystems, CA, U.S.A.).

Sequencing of PCR products

Once a subset of PCR products with an aberrant CSGE pattern had been identified, the PCR products were re-amplified using the same primers as for CSGE and subjected to DNA sequencing in both directions on an ABI3700 genetic analyzer (Applied Biosystems) (Fig. 1).

Interpretation of sequence analysis results

The sequence analysis results were sorted into three categories. *Positive for BRCA mutation* included nonsense or frameshift mutations that produce prematurely terminated (truncated) protein products of *BRCA1* or *BRCA2*, whose clinical significance has been determined. *Unverified mutations (UVs)* included missense mutations and mutations that occurred in analyzed intronic regions whose clinical significance has not yet been determined. *Negative for BRCA mutation* included samples identical to a consensus wild-type sequence as well as samples in which genetic variants of no substantial clinical consequence were identified.

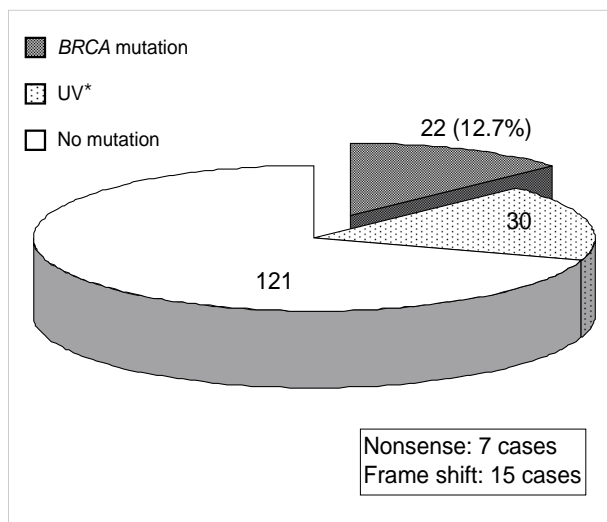


Fig. 2. BRCA mutations in breast cancer patients with high-risk factors in Korea (n=173). \*UV, unverified mutation.

RESULTS

In 109 unselected breast cancer patients, 3 individuals (2.8%) carried *BRCA* mutations, two in *BRCA1* and one in *BRCA2*; of these, one was a nonsense mutation and two were frame shift mutations. In contrast, 22 of the 173 high-risk breast cancer patients (12.7%) carried *BRCA* mutations, 15 (8.7%) in *BRCA1* and seven (4%) in *BRCA2*; of these, seven were nonsense mutations and 15 were frame shift mutations (Fig. 2).

Among the 5 subsets of high risk patients, mutations were most prevalent in patients having a family history of breast or first-degree ovarian cancer (22.1%), followed by those with male breast cancer (20%), bilateral breast cancer (20%), multiple organ cancer including breast cancer (13%) and younger breast cancer patients (aged <35 yr) (8.1%) (Table 1).

We found that *BRCA* mutations were present in 8 of 23 patients (34.8%) with two high-risk factors. *BRCA1* mutations were detected in 3 of 14 patients (21.4%) with family history and early-onset (<35 yr), one of 2 patients (50%) with family history and bilateral cancer, one of 2 patients (50%) with family history and male breast cancer, one of 2 patients (50%) with family history and multi-organ cancer, one of 2 patients (50%) with early-onset and bilateral cancer, and none of one patient with early-onset and multi-organ cancer. *BRCA2* mutation was studied in 14 patients with family history and early-onset (<35 yr) and detected in one of the 14 patients (7.1%).

DISCUSSION

Mutations in the *BRCA1* and *BRCA2* genes in females confer highly elevated risks of developing cancers of the breast and ovary. Ever since the *BRCA1* and *BRCA2* genes were identified in 1994 and 1995, respectively, there has been controversy over whether presymptomatic testing for these mutations would be clinically beneficial, and, if so, who would be eligible for testing (13). Since the prevalence of *BRCA1* and *BRCA2* mutations in most populations is low, many advisory bodies, including one under the auspices of the American Society for Clinical Oncology, have recommended that testing be restricted to women at high risk of developing breast or ovarian can-

Table 1. BRCA mutations in high-risk patients with breast cancer in Korea

	Family history of breast or first-degree ovarian cancer	Bilateral breast cancer	Male breast cancer	Multiple organ cancer	Earlier age of onset (<35 yr)	Total
Cases tested	86	15	5	16	74	173*
<i>BRCA1</i> mutation	13 (15.1%)	2 (13%)	1 (20%)	2 (13%)	5 (6.8%)	15 (8.7%)
<i>BRCA2</i> mutation	6 (7%)	1 (6.7%)	-	-	1 (1%)	7 (4%)
Total (%)	19 (22.1%)	3 (20%)	1 (20%)	2 (13%)	6 (8.1%)	22 (12.7%)

\*Of total 173 cases, 23 cases have two high-risk factors.

cer (14).

At present, hundreds of *BRCA* mutations are known. Most of these are nonsense or frame shift mutations, which can be found throughout the entire gene sequence and produce truncated proteins (15). Due to founder effects as well as other environmental and geographical factors, the prevalence of *BRCA* mutations is variable among different populations (10, 11).

In this report, we evaluated the prevalence of *BRCA1* and *BRCA2* mutations in high-risk patients as well as in unselected breast cancer patients in Korea. We found that the prevalence of these mutations was about 4-5 times higher in high-risk patients than in unselected patients. To our knowledge, this is the first population-based report of *BRCA* mutations in breast cancer patients in Korea. Interestingly, however, we did not detect any of the three founder mutations identified in individuals of Ashkenazi Jewish descent (185delAG and 5382 insC for *BRCA1* and 6174delT for *BRCA2*) or the 999del5 mutation for *BRCA2* common to the Icelandic population (6) in any Korean patient.

We found that a family history of breast or first-degree ovarian cancer was the most important high-risk factor for *BRCA* mutation, followed by bilateral breast cancer and male breast cancer. Each of these factors increased the likelihood of detecting *BRCA* mutations 7- to 8-fold compared with the unselected patients. In contrast, earlier age at onset, prior to age 35, increased the likelihood of *BRCA* mutations 3-fold compared with the unselected patients. Our finding of *BRCA* mutations in 23% of Korean patients with a family history of breast cancer is in good agreement with findings in Caucasian and other Asian patients, in whom *BRCA* mutations were detected in 11% to 33% of those with a family history of breast cancer (2, 19, 20, 24). In some of these studies, 45% to 51% of patients with two or more relatives with breast cancer were found to have *BRCA* mutations (20, 24).

It has been reported that nine of 21 (43%) Korean families containing two or more affected first- or second-degree relatives with breast and/or ovarian cancer had *BRCA* germline mutations (four frameshift and five nonsense) (9). In our study, we detected 15 frameshift mutations and seven nonsense mutations.

There are few reports on the prevalence of *BRCA* gene mutations in unselected breast cancer patients in Asia. The prevalence of these mutations has been estimated at 5.1% to 6.7%, with the phenotypes varying by ethnicity (2, 16). In our study of unselected Korean breast cancer patients, however, we detected a lower prevalence of *BRCA* mutations (2.8%), which may be due to ethnic differences. It should be noted, however, that the numbers of unselected Asian breast cancer patients canvassed to date is low; larger numbers may give a more accurate estimate of the frequency of *BRCA* mutations in Asia, as well as of the variations between Asian populations.

The prevalence of *BRCA* mutations in patients with early-onset breast cancer is quite different depending on ethnicity. For example, this rate has been found to be 20-30% in Ashke-

nazi Jewish and Icelandic patients (7, 17, 18), less than 10% in Caucasian patients (19-22), and 6-11% in Asian patients, including our results (2, 23).

We found that the prevalence of *BRCA* mutations in patients with two or more risk factors was 2.7 times higher than that in patients with a single risk factor. These risk factors included early age at diagnosis, bilateral cancer, multiple organ cancer, and male breast cancer, together with a family history of breast or first-degree ovarian cancer. For example, 28.6% of patients diagnosed prior to age 35 yr and who had a family history of breast or first-degree ovarian cancer carried *BRCA* mutations, a prevalence comparable to the 29.7% of non-Ashkenazi breast cancer patients diagnosed before age 40 yr who had one relative diagnosed with breast cancer before age 50 (24). In this latter report, 50.7% of these patients who had two relatives diagnosed with breast cancer before age 50 carried germline *BRCA* mutations (24).

We detected *BRCA* mutations in 50% of patients with bilateral cancer, multiple organ cancer, or male breast cancer and a familial history, and with bilateral cancer diagnosed below age 35, although the number of patients in each category was small. Larger, population-based studies are needed to evaluate the impact of these combined risk factors on the prevalence of *BRCA* mutations. Interestingly, it has been reported that a family history of bilateral breast cancer did not substantially increase the likelihood of *BRCA* mutations (19). Previous investigations have suggested that male breast cancer may be another hallmark for the presence of *BRCA2* mutations (19, 25, 26). In contrast, of the small number (5) of male breast cancer patients that we assayed, none had a *BRCA2* mutation, and only one had a *BRCA1* mutation.

*BRCA1* mutations have been found to be significantly more common in women with a family history of ovarian cancer than in those with a family history of breast cancer (19). In contrast, *BRCA2* mutations were not found to be more common in women with a family history of ovarian cancer. In Asian countries, *BRCA* mutations have been detected in 4.7% to 15.8% of patients with ovarian cancer (16, 27). While the prevalence of *BRCA* mutations has been shown to be increased in patients with earlier onset breast cancer, the prevalence of these mutations was no higher in women with ovarian cancer diagnosed before age 50 than in those diagnosed at a later age (24).

We believe that our data on the personal and family histories of the individuals tested are accurate because the information was obtained from the test requisition form by a research fellow. Moreover, the F-CSGE technique we used has been found to detect 98% of *BRCA* mutations, a sensitivity at least equal to that of other gel-based genomic screening techniques (28).

Generally, tests for *BRCA* mutations are recommended in cases with 10% or higher prevalence rate of the mutation in a clinical setting. Our study data suggest that breast cancer patients with high-risk factors, including a family history of

breast or first-degree ovarian cancer, bilateral cancer, male breast cancer, multiple organ cancer, and earlier age at onset, who have a higher prevalence of *BRCA* mutations, should be tested for *BRCA* mutations. Our finding, that the phenotype of *BRCA* mutations in Korea is different from those of Ashkenazi Jew and other populations, suggests the need for large-scale, population-based studies to establish the clinicopathologic characteristics as well as the genetic pathology related to *BRCA* mutations in Korean breast cancer patients.

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