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SHORT COMMUNICATION

Spectra of *BRCA1* and *BRCA2* mutations in Korean patients with breast cancer: the importance of whole-gene sequencing

This article has been corrected since advance Online Publication, and a corrigendum is also printed in this issue

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The frequencies and spectra of germline mutations in the *BRCA1* and *BRCA2* genes vary among populations. In the present study, the mutation spectra of the *BRCA1/BRCA2* genes in Korean breast cancer patients were investigated using whole-gene sequencing method. A total of 134 unrelated Korean breast cancer patients who were identified as being at high risk of carrying *BRCA1/BRCA2* mutations were included. PCR amplification and direct sequencing were performed covering all exons and flanking intronic sequences of the *BRCA1/BRCA2* genes. A total of 26 mutations were detected in 31 of 134 patients (23.1%). The mutation detection rate in the present study is higher than those of previous studies using screening methods (2.5–11.3%) and similar to that of a recent study, which used whole-gene sequencing (21.2%). The *BRCA2*: c.7480C>T mutation, which has been suggested to be a founder mutation in Koreans, was detected in only one patient. Five mutations were recurrent but observed in no more than two patients. Given that the mutation detection rates using whole-gene sequencing were much higher than for screening methods and that there were no consistent observations of founder mutations, whole-gene sequencing of both *BRCA1* and *BRCA2* genes should be the method of choice to identify mutations in high-risk Korean patients.
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Keywords: *BRCA1*; *BRCA2*; breast neoplasms; Korean; sequence analysis

INTRODUCTION

A wide variety of germline mutations in either *BRCA1* or *BRCA2 (BRCA1/BRCA2)* genes are responsible for the majority of hereditary breast and ovarian cancers. The identification of mutations in the *BRCA1/BRCA2* genes is necessary for genetic counseling and to consider risk-reduction strategies in high-risk family members.[1]

The incidence of breast cancer in Korea has continuously increased in recent years.[2] Studies of breast cancer epidemiology in samples of Asian and European descent have found differences between populations, potentially due to the interactions between different lifestyle and genetic characteristics.[3,4] As genetic predispositions to breast cancer are increasingly understood, it has been suggested that there are differences between populations and that molecular diagnostic strategies should be important components of treatment. The discovery of founder mutations might lead to cost-effective options to screen high-risk families, as in Ashkenazi Jews.[5]

To date, only a few studies have examined the frequencies and spectra of mutations in large series of Korean breast cancer patients.[6–10] Several mutations are considered to be founder mutations, as they account for the majority of detected *BRCA1/BRCA2* mutations in Korea. Although some of these recurrent mutations are unique to Koreans, their effects vary across studies. In the present study, we investigated the frequencies and spectra of *BRCA1/BRCA2* gene mutations in Korean breast cancer patients to identify common mutations and to establish an optimal molecular diagnostic strategy.

MATERIALS AND METHODS

A total of 277 patients who were diagnosed with breast and/or ovarian cancer in Samsung Medical Center were requested for *BRCA1/BRCA2* mutation analysis during September 2001 to December 2009. Informed consent was obtained from all patients for clinical information and genetic analysis. The study was conducted in accordance with the Declaration of Helsinki of 1975, as revised in

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Table 1 *BRCA1* and *BRCA2* mutations in Korean patients with breast cancer

Exon/intron	*Nucleotide change*[a]	*BIC nomenclature*	*Amino-acid change*[a]	*Citation*	*Risk factors*
BRCA1					
IVS6	c.301-2A>C	420-2A>C	—	BIC	M (1)
7	c.390C>A	509 C>A	p.Y130X	BIC	F (1)
11	c.922_924delAGCinsT	1041_10423delAGCinsT	p.S308X	[b]	FM (1)
11	**c.1511dupA**	**1610dupG**	**p.K505X**	**Novel**	**MF (1)**
11	**c.1936delA**	**2055delA**	**p.S646AfsX5**	**Novel**	**F (1)**
11	c.3627dupA	3746dupA	p.E1210RfsX9	BIC	FM (1)/EF (1)
11	**c.3814dupT**	**3933dupT**	**p.N1272X**	**Novel**	**FB (1)**
IVS18	c.5152+1G>C	5271+1G>C	—	BIC	EF (1)
IVS21	**c.5278-2A>T**	**5397-2A>T**	**—**	**Novel**	**F (1)**
23	c.5445G>A	5564G>A	p.W1815X	BIC	F (2)
24	c.5496_5506del11insA	5615_5625del11insA	p.V1833SfsX7	[b]	F(1)/EF(1)
BRCA2					
3	c.97G>T	325G>T	p.E33X	[c,d]	EF (1)/FB (1)
3	**c.196C>T**	**424C>T**	**p.Q66X**	**Novel**	**F (1)**
10	c.1310_1313delAAGA	1538_1541delAAGA	p.K437IfsX22	BIC	EF (1)
10	**c.1514delT**	**1742delT**	**p.I505NfsX4**	**Novel**	**F (1)**
11	**c.3018delA**	**3246delA**	**p.G1007VfsX36**	**Novel**	**EF (1)**
11	c.3744_3747delTGAG	3792_3975delTGAG	p.S1248RfsX10	BIC	BF (1)
11	c.4766delC	4994delC	p.P1589QfsX28	[b]	BEF (1)
11	**c.5116_5119delAATA**	**5344_5347delAATA**	**p.N1706LfsX5**	**Novel**	**FM (1)**
11	**c.5574_5577delAATT**	**5802_5805delAATT**	**p.I1859KfsX3**	**Novel**	**F (1)**
11	**c.6723_6724delAG**	**6951_6952delAG**	**p.D2242FfsX2**	**Novel**	**BE (1)**
13	c.6952C>T	7180C>T	p.R2318X	BIC	F (1)/EF (1)
14	**c.7258G>T**	**7486G>T**	**p.E2420X**	**Novel**	**F (1)**
15	c.7480C>T	7708G>T	p.R2494X	BIC	F (1)
IVS20	**c.8633-2A>T**	**8861-2A>T**	**—**	**Novel**	**EF (1)**
23	c.9117G>A	9345G>A	Aberrant splicing	[e]	F (1)

Novel mutations are shown in bold.
[a]Numbers are based on the complementary DNA sequence with A of ATG translation-initiation codon as +1.
[b]Reported by Seo *et al.*[7]
[c]Reported by Seong *et al.*[10]
[d]Reported by Capalbo *et al.*[15] (Ann Oncol. 17 suppl 7, vii34–40 (2006)).
[e]Reported by Whiley *et al.*[16] (BMC Med Genet. 11, 80 (2010)).

2000. Selection of patients was based on the following criteria as previously described[9,10] and at least one of the following: (F) at least one first- or second-degree relative with breast and/or ovarian cancer diagnosed at any age, (E) early-onset breast cancer diagnosed at age 35 or before, (B) bilateral breast cancer and (M) multiple organ cancer, including breast cancer. The study subjects consisted of 134 unrelated Korean breast cancer patients who were identified as being at high risk of carrying *BRCA1/BRCA2* mutations; 116 (87%) familial cases, 29 (22%) early-onset cancer cases, 13 (10%) bilateral cancer cases and 10 (7%) multiple organ cancer cases. There were no male breast cancer cases in our sample. The mean age at diagnosis was 43 years (range 22–66). A total of 96 healthy Korean subjects, who visited for regular health checkup, were screened as a control for novel sequence variations.

PCR and direct sequencing were performed covering whole exons and flanking intronic sequences of the *BRCA1/BRCA2* genes with primers designed by the authors (available upon request). Reference sequences for the *BRCA1* and *BRCA2* genes were GenBank accession numbers NM_007294.2 and NM_000059.3, respectively. For the descriptions of the variations identified, we followed the nomenclature system of the Human Genome Variation Society (http://www.hgvs.org/mutnomen) and the conventional nomenclature system from the Breast Cancer Information Core (BIC; http://research.nhgri.nih.gov/bic/). *In silico* prediction of functional consequences of missense variation was performed using SIFT (Sorting Intolerant From Tolerant; http://sift.bii.a-star.edu.sg/),[11] PolyPhen (Polymorphism Phenotyping, http://genetics.bwh.harvard.edu/pph2/),[12] PANTHER (Protein analysis through Evolutionary Relationships, http://www.pantherdb.org)[13] and SVM (Support Vector Machine).[14]

RESULTS

A total of 26 mutations for the *BRCA1* or *BRCA2* genes were identified in 31 of 134 unrelated Korean patients (23.1%, 31/134) (Table 1). According to mutation type, frameshift mutations were most frequent (n=11), followed by nonsense mutations (n=10) and splicing mutations (n=5). Twelve of 26 distinct mutations were novel; they had not previously been reported to the BIC database or described in publications, to our knowledge. Five recurrent mutations (*BRCA1*: c.3627dupA, c.5445G>A and c.5496_5506del11insA; *BRCA2*: c.97G>T and c.6952C>T) were found in the present study, but were observed in no more than two patients and accounted for 32.3% (10/31) of the *BRCA1/ BRCA2* mutations.

During sequencing analysis, we also detected 13 other sequence variations (9 in *BRCA1* and 4 in *BRCA2*), which were missense variations of unknown significance within the coding regions (Table 2). These variations have not been reported in the SNP database (http://www.ncbi.nlm.nih.gov/snp) and eight have been reported in the BIC database but are of unknown clinical importance. Among the 13 unclassified variations, 11 variations have been found in previous normal control studies[8,10] and/or in the present study, but there is not reliable functional assay at present. Four different protein prediction programs (PolyPhen, SIFT, PANTHER and SVM) were used to predict the functional consequences of the mutations and obtain diverse results.

Table 2 Missense variations of unknown significance in *BRCA1* and *BRCA2* genes

					Number of cases						
Exon	*Nucleotide change*[a]	*BIC nomenclature*	*Amino-acid change*[a]	*BIC*	*Patient*	*Control study*		*PolyPhen*	*SIFT*	*PANTHER*	*SVM*
BRCA1											
5	c.154C>T	273C>T	p.L52F	Unknown	3	2/96	0/167[b]	Probably	Intolerant	Deleterious	Damaging
11	c.2566T>C	2685T>C	p.Y856H	Unknown	2	9/96	—	Benign	Intolerant	Deleterious	Damaging
11	c.2612C>T	2731C>T	p.871L	—	1	24/96	—	Benign	Tolerated	Not deleterious	Benign
11	c.2726A>T	2845A>T	p.N909I	—	1	0/96	—	Benign	Intolerant	Not deleterious	Damaging
11	c.3448C>T	3567C>T	p.P1150S	Unknown	2	2/96	—	Possibly	Intolerant	Not deleterious	Damaging
11	c.3650C>G	3769C>G	p.S1217C	—	1	9/96	—	Probably	Intolerant	Deleterious	Damaging
16	c.4729T>C	4848T>C	p.S1577P	Unknown	2	0/96	3/167[b]	Possibly	Intolerant	Deleterious	Damaging
16	c.4883C>T	5002T>C	p.M1628T	Unknown	1	2/96	3/167[b]	Possibly	Tolerated	Not deleterious	Damaging
22	c.5339T>C	5458T>C	p.L1780P	Unknown	1	2/96	3/167[b]	Probably	Intolerant	Deleterious	Damaging
BRCA2											
11	c.5969A>G	6197A>C	D1990A	—	1	0/96	1/171[c]	Probably	Intolerant	Deleterious	Damaging
17	c.7814G>A	8042G>A	C2605Y	—	1	2/96	—	Probably	Intolerant	Deleterious	Damaging
18	c.8187G>T	8415G>T	K2729N	Unknown	1	2/96	—	Benign	Intolerant	Not deleterious	Damaging
26	c.9590A>G	9818A>G	D3197G	—	1	0/96	—	Probably	Intolerant	Deleterious	Damaging

Abbreviations: BIC, Breast Cancer Information Core; PANTHER, Protein Analysis Through Evolutionary Relationship; PolyPhen, Polymorphism Phenotyping; SIFT, Sorting Intolerant From Tolerant; SVM, Support Vector Machine.
[a]Numbers are based on the complementary DNA sequence with A of ATG translation-initiation codon as +1.
[b]Reported by Ahn *et al.*[9]
[c]Reported by Seong *et al.*[10]

Table 3 *BRCA1* and *BRCA2* mutation detection rates in Koreans reported in previous studies

Study	*Inclusion criteria*	*Assay method*	*Mutation frequency*
Choi *et al.*[6]	Younger cases (⩽40 years)	Whole-gene sequencing	15.0% (9/60)
Seo *et al.*[7]	Sporadic cases	F-CSGE and sequencing	3.1% (3/97)
Han *et al.*[8]	Sporadic cases	DHPLC and sequencing	2.5% (20/793)
Ahn *et al.*[9]	High-risk group[a]	F-CSGE and sequencing	11.3% (40/354)
Seong *et al.*[10]	High-risk group[b]	Whole-gene sequencing	21.2% (33/156)
This study	High-risk group[b]	Whole-gene sequencing	23.1% (31/134)

Abbreviations: DHPLC, denaturing high-performance liquid chromatography; F-CSGE, fluorescent-conformation sensitive gel electrophoresis.
[a]At least two incidences of family history.
[b]At least one incidence of family history.

DISCUSSION

There have been two reports of common mutations of the *BRCA1/BRCA2* genes in Korean breast cancer patients who were identified as being at high risk of carrying *BRCA1/BRCA2* mutations. Ahn *et al.*[9] reported that the combination of seven common mutations (*BRCA1*: c.2433delC, c.3627dupA, c.4065_4068delTCAA, c.5470_5477del8 and c.5496_5506del11insA; *BRCA2*: c.1399A>T and c.7480C>T) accounted for 62.5% and this suggests the possibility of founder effect. Seong *et al.*[10] analyzed data from previous two large studies[7,9] and reported that another set of seven common mutations (*BRCA1*: c.390C>A, c.2433delC, c.3627dupA, c.4065_4068delTCAA and c.5496_5506del11insA; *BRCA2*: c.3744_3747delTGAG and c.7480C>T) was recurrent and accounted for up to 43.9% in the pooled population. Adding the results of our series, the mutations with frequencies over 3.0% in the pooled dataset were as follows; *BRCA1*: c.390C>A (3.1%), c.3627dupA (6.9%) and c.5496_5506del11insA (4.6%); *BRCA2*: c.1399A>T (3.1%), c.3744_3747delTGAG (4.6%) and c.7480C>T (13.9%), and seven most common mutations accounted for 38.7% (50/129). Interestingly, the most common mutation (*BRCA2*: c.7480C>T) in the previous studies, where the proportion of the mutation ranged from 15 to 20%, was detected in only one patient (3.2%, 1/31). Considering the limited number of mutation-positive patients in an individual study or a pooled dataset, additional research is required to determine the founder effect on Korean population.

Most previous studies of *BRCA1/BRCA2* genes in Korea used performed two-step approaches, in which mutations are first scanned in a rapid manner, and then subjected to sequencing analysis when sequence alterations are detected.[6–10] Table 3 shows the mutation detection rates in the *BRCA1/BRCA2* genes from each study in Korea. Compared with the two studies using similar inclusion criteria for the high-risk group of Korean breast cancer patients, mutation detection rates of whole-gene sequencing exceeded 20%. In contrast, a two-step approach with a combination of fluorescent-conformation sensitive gel electrophoresis and sequencing analysis resulted in a lower mutation detection rate (11.3%), although more strict inclusion criteria for family history (at least two patients with breast and/or ovarian cancer) was applied.

In the present study, we identified 26 mutations in *BRCA1/BRCA2* genes in 31 of 134 (23.1%) unrelated Korean patients who were identified as being at high risk of carrying *BRCA1/BRCA2* mutations. Given that the rates of mutation detection using whole-gene sequencing are much higher than those of the two-step screening method

and that we did not observe founder mutations, whole-gene sequencing of both *BRCA1* and *BRCA2* genes should be the method of choice used to identify mutations in high-risk Korean patients.

1 Uyei, A., Peterson, S. K., Erlichman, J., Broglio, K., Yekell, S., Schmeler, K. *et al.* Association between clinical characteristics and risk-reduction interventions in women who underwent BRCA1 and BRCA2 testing: a single-institution study. *Cancer* **107,** 2745–2751 (2006).
2 Ko, S. S. Chronological changing patterns of clinical characteristics of Korean breast cancer patients during 10 years (1996–2006) using nationwide breast cancer registration on-line program: biannual update. *J. Surg. Oncol.* **98,** 318–323 (2008).
3 Fackenthal, J. D. & Olopade, O. I. Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. *Nat. Rev. Cancer* **7,** 937–948 (2007).
4 Li, C. I., Malone, K. E. & Daling, J. R. Differences in breast cancer hormone receptor status and histology by race and ethnicity among women 50 years of age and older. *Cancer Epidemiol. Biomarkers Prev.* **11,** 601–607 (2002).
5 Abeliovich, D., Kaduri, L., Lerer, I., Weinberg, N., Amir, G., Sagi, M. *et al.* The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women. *Am. J. Hum. Genet.* **60,** 505–514 (1997).
6 Choi, D. H., Lee, M. H., Bale, A. E., Carter, D. & Haffty, B. G. Incidence of BRCA1 and BRCA2 mutations in young Korean breast cancer patients. *J. Clin. Oncol.* **22,** 1638–1645 (2004).
7 Seo, J. H., Cho, D. Y., Ahn, S. H., Yoon, K. S., Kang, C. S., Cho, H. M. *et al.* BRCA1 and BRCA2 germline mutations in Korean patients with sporadic breast cancer. *Hum. Mutat.* **24,** 350 (2004).
8 Han, S. H., Lee, K. R., Lee, D. G., Kim, B. Y., Lee, K. E. & Chung, W. S. Mutation analysis of BRCA1 and BRCA2 from 793 Korean patients with sporadic breast cancer. *Clin. Genet.* **70,** 496–501 (2006).
9 Ahn, S. H., Son, B. H., Yoon, K. S., Noh, D. Y., Han, W., Kim, S. W. *et al.* BRCA1 and BRCA2 germline mutations in Korean breast cancer patients at high risk of carrying mutations. *Cancer Lett.* **245,** 90–95 (2007).
10 Seong, M. W., Cho, S., Noh, D. Y., Han, W., Kim, S. W., Park, C. M. *et al.* Comprehensive mutational analysis of BRCA1/BRCA2 for Korean breast cancer patients: evidence of a founder mutation. *Clin. Genet.* **76,** 152–160 (2009).
11 Kumar, P., Henikoff, S. & Ng, P. C. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat. Protoc.* **4,** 1073–1081 (2009).
12 Sunyaev, S., Ramensky, V., Koch, I., Lathe, W. III, Kondrashov, A. S. & Bork, P. Prediction of deleterious human alleles. *Hum. Mol. Genet.* **10,** 591–597 (2001).
13 Thomas, P. D., Campbell, M. J., Kejariwal, A., Mi, H., Karlak, B., Daverman, R. *et al.* PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res.* **13,** 2129–2141 (2003).
14 Won, H. H., Kim, H. J., Lee, K. A. & Kim, J. W. Cataloging coding sequence variations in human genome databases. *PLoS One* **3,** e3575 (2008).
15 Capalbo, C., Ricevuto, E., Vestri, A., Ristori, E., Sidoni, T., Buffone, O. *et al.* BRCA1 and BRCA2 genetic testing in Italian breast and/or ovarian cancer families: mutation spectrum and prevalence and analysis of mutation prediction models. *Ann Oncol* **17**(Suppl 7)**,** vii34–vii40 (2006).
16 Whiley, P. J., Pettigrew, C. A., Brewster, B. L., Walker, L. C., Spurdle, A. B. & Brown, M. A. Effect of BRCA2 sequence variants predicted to disrupt exonic splice enhancers on BRCA2 transcripts. *BMC Med Genet* **11,** 80 (2010).