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Novel Germline Mutations of *BRCA1* and *BRCA2* in Korean Familial Breast Cancer Patients

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Breast cancer is the second most common cancer in Korean women. Germline mutations in the *BRCA1* and *BRCA2* genes cause hereditary breast cancer and are detected in 15-20% of hereditary breast cancer. We investigated the *BRCA1* and *BRCA2* mutations in 114 familial breast cancer patients using next-generation sequencing. We confirmed 20 different mutations of *BRCA1* and *BRCA2* in 25 subjects (21.9%). Two such mutations in eight patients were novel (not reported in any variant database or previous study). Six mutations have been reported as disease-causing mutations in public databases. Seven mutations were found only in a single nucleotide polymorphism database and one mutation has been reported in Korea. The *BRCA1/2* mutation frequency was similar to that of other studies on familial breast cancer patients in the Korean population. Further studies should examine more cases and mutations of whole exons.

Key Words: *Breast Neoplasms; Mutation; BRCA1 Protein; BRCA2 Protein*

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INTRODUCTION

Breast cancer has been the second most common cancer in Korean women since 2004. In 2013, 19,316 patients were newly diagnosed with breast cancer and the crude incidence was 76.2 cases per 100,000 women.[1] The median age at diagnosis is 50 years, and more than half of all cases are diagnosed in postmenopausal women. However, the proportion of early breast cancer has consistently increased.[2]

BRCA1 and *BRCA2* are tumor suppressor genes that are inherited in an autosomal dominant pattern. Germline mutations of these two highly penetrant genes cause hereditary breast cancer[3,4] and are detected in 15-20% of hereditary breast cancer cases.[5] The reported lifetime risk of breast cancer with mutations in the *BRCA1/2* genes is between 60% and 85%.[6,7] The management of breast cancer patients differs depending on whether a mutation is present or not.[8,9]

The prevalence of *BRCA* mutations varies with ethnicity and country.[10,11] Many studies have examined the prevalence of *BRCA1* and *BRCA2* mutations in Korean breast cancer patients,[12-14] including two recent studies[12,14] that reported that the prevalence of *BRCA* mutations among familial breast cancer patients was 19.6% and 20.60%, respectively. However, studies of more patients are still needed to evaluate the prevalence of *BRCA* mutations in Korean breast cancer patients.

In this study, we investigated the prevalence of *BRCA1* and *BRCA2* mutations and explored the novel variants among the familial breast cancer patients in Korea.

MATERIALS AND METHODS

1. Subjects

All patients were enrolled at Chonnam National University Hwasun Hospital (Hwasun, Korea) between 2005 and 2012. From 3,540 female breast cancer patients, we selected 161 patients (4.6%) with a family history of breast cancer. After excluding 47 patients with low quantity and quality of genomic DNA for library preparation with the polymerase chain reaction (PCR)-based Access Array, 114 breast cancer patients were included in the analyses, including 16 patients younger than 40 years old, 6 smokers, and 11 obese patients with a body mass index ≥ 30 kg/m^2. Information on the family history of breast cancer and other risk factors was collected via review of medical records. Study participants agreed with the informed consent form

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and this study was approved by the Institutional Review Board of Chonnam National University Hwasun Hospital (HCRI-09-043-3).

2. Mutation detection

Genomic DNA was extracted from peripheral blood using a QIAamp DNA Mini Kit (QIAGEN, Chatsworth, CA, USA), according to the manufacturer's protocol.

The procedure entailed the design of target-specific primers, samples preparation, and then running a PCR-based Access Array (Fluidigm, San Francisco, CA, USA) and qualifying and quantifying harvested PCR products for sequencing. Briefly, 184 amplicons were designed to cover all exons of *BRCA1* and *BRCA2*. Samples were amplified using the 48.48 PCR-based Access Array (Fluidigm) and purified pooled samples were sequenced on a MiSeq sequencer (Illumina, Hayward, CA, USA), according to the manufacturers' protocols.

3. Data analysis

The data was analyzed on a PCR Amplicon workflow with MiSeq Reporter software ver. 2.4.60 (Illumina, Hayward, CA, USA), which used the Burrows–Wheeler Aligner. Reads were aligned against the GRCh37/ hg19 reference genomes of targeted regions in the sample sheet. Integrative Genomics Viewer was used to visualize the quality and variance of the Bam and VCF files.[15]

Variants with a minor allele frequency >1% in Ensemble and synonymous and intronic variants located outside the exon/intron boundaries were excluded from further analysis. To classify pathogenic mutations, all nonsynonymous variants were compared to a *BRCA* locus-specific database (http://research.nhgri.nih.gov/bic/, http://databases.lovd.nl/shared/genes/*BRCA1*, and http://databases.lovd.nl/shared/genes/*BRCA2*) and The Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php) and amino acid variation was predicted using the predictive algorithms SIFT, PolyPhen-2, and Mutation T@ster web ser-

TABLE 1. The clinical characteristics of the study participants

	Total (n=114)	*BRCA1/2* (n=25)	*BRCA1* (n=10)	*BRCA2* (n=15)
Age at diagnosis (years)	51.7 (30-78)	51.8 (34-73)	50.6 (36-67)	52.8 (34-73)
Early onset (≤35 years)	6 (5.3)	1 (4.0)	0 (0.0)	1 (6 .7)
Mean BMI (kg/m^2)	24.2 (3.58)	24.0 (2.41)	23.2 (2.47)	24.5 (2.31)
High BMI (≥25 kg/m^2)	36 (31.9)	8 (32.0)	2 (20.0)	6 (40.0)
Stage at diagnosis				
0-I	45 (39.5)	6 (24.0)	3 (30.0)	3 (20.0)
II	44 (37.7)	9 (46.7)	4 (40.0)	5 (33.4)
III	22 (19.3)	10 (40.0)	3 (30.0)	7 (46.7)
IV	3 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)
Relatives with breast cancer				
1st - degree	30 (26.3)	9 (35.0)	2 (20.0)	7 (46.7)
2nd - degree	62 (54.4)	11 (44.0)	4 (40.0)	7 (46.7)
3rd - degree	5 (4.4)	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	17 (14.9)	6 (24.0)	4 (40.0)	2 (13.3)
Smoking habit				
Never	108 (94.7)	24 (96.0)	10 (100.0)	14 (93.3)
Ever	6 (5.3)	1 (4.0)	0 (0.0)	1 (6.7)
Alcohol drinking				
Never	88 (77.2)	17 (68.0)	6 (60.0)	11 (73.3)
Ever	24 (21.1)	8 (32.0)	4 (40.0)	4 (26.7)
Unknown	2 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)
Estrogen receptor				
Positive	68 (59.6)	14 (56.0)	3 (30.0)	11 (73.3)
Negative	44 (38.6)	11 (44.0)	7 (70.0)	4 (26.7)
Unknown	2 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)
Progesteron receptor				
Positive	68 (59.6)	15 (60.0)	4 (40.0)	11 (73.3)
Negative	44 (38.6)	10 (40.0)	6 (60.0)	4 (26.7)
Unknown	2 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)
Menopausal status				
Pre-menopause	60 (52.6)	13 (52.0)	6 (60)	7 (46.7)
Post-menopause	53 (46.5)	12 (48.0)	4 (40)	8 (53.3)
Unknown	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)

Values; count (range), mean (standard deviation), and count (%), as appropriate.

TABLE 2. *BRCA1* and *BRCA2* mutations in breast cancer patients with family history

Gene	Exon	Mutations	Mutation type	PolyPhen-2	SIFT	Mutation taster	N	Database
BRCA1	7	Tyr130Ter	stop gained			disease causing	1	rs80356888, HGMD, BIC, KS
	11	Gly275Asp	missense	possibly damaging	deleterious	polymorphism	1	rs397509327, HGMD
	11	Val409Leu	missense	possibly damaging	tolerated	polymorphism	1	rs144698700
	11	Tyr655ValfsTer18	frameshift			disease causing	1	rs80357853
	11	Pro1150Ser	missense	probably damaging	deleterious	disease causing	2	rs80357272, HGMD, BIC
	17	c.5074_5074+4delGGTAT	splicing			disease causing	1	novel
	22	Leu1780Pro	missense	benign	deleterious	disease causing	2	rs80357474, HGMD, BIC
	22	Thr1802Pro	missense	possibly damaging	deleterious	polymorphism	1	novel
BRCA2	10	c.1286_1287insA	frameshift			disease causing	2	novel
	11	Glu1113Ter	nonsense			disease causing	1	novel
	11	Ala1393Val	missense	benign	tolerated	polymorphism	1	rs398122776
	11	Lys1440Asn	missense	probably damaging	deleterious	disease causing	1	rs769535925
	11	Ser1632Asn	missense	benign	tolerated	polymorphism	1	novel
	11	Tyr1710Asp	missense	benign	tolerated	polymorphism	1	novel
	11	Asp1864Asn	missense	benign	deleterious	polymorphism	1	rs587781536
	13	Arg2336Alafs (7006delC)	splicing			disease causing	1	KS
	14	Leu2396Phe	missense	possibly damaging	tolerated	polymorphism	2	rs587780871
	15	Arg2494Ter	stop gained	-	-	disease causing	1	rs80358972, HGMD,KB, BIC
	23	Gln3026Glu	missense	possibly damaging	deleterious	disease causing	2	rs80359159, BIC
	27	Gln3398Arg	missense	benign	tolerated	polymorphism	1	rs374275215

HGMD: the Human Gene Mutation database, BIC: the Breast Cancer Information Core, KS: Korean Study.

vers.[16-18] All variants in this study were classified as pathogenic mutations or variants of uncertain clinical significance (VUCS), as previously described.[19] All rare variants were confirmed by conventional Sanger sequencing and high-resolution melting analysis.

RESULTS

Table 1 shows the clinical characteristics of the study participants. The mean age at diagnosis of breast cancer was 51.7 (range 29.7-77.9) years. The proportions of smokers, alcohol drinkers, estrogen-receptor-positive, progesterone-receptor-positive, and menopausal subjects among the all breast cancer subjects and subjects with *BRCA* mutations are also presented. No subjects had a history of ovarian cancer (Table 1).

Table 2 shows the distribution of mutations in *BRCA1* and *BRCA2*. We confirmed 20 different *BRCA1/2* mutations in 25 (21.9%) of the 114 patients. Eight of the mutations were in the *BRCA1* gene and twelve were in the *BRCA2* gene. Six mutations (in nine patients) have been reported as disease-causing mutations in public databases (BIC and HGMD). Two *BRCA1* mutations and five *BRCA2* mutations were found only in a single nucleotide polymorphism database and one mutation had been reported in a Korean. Two of the *BRCA1* and four of the *BRCA2* mutations in eight patients were novel mutations (not reported in any variant database or previous study).

Interestingly, three novel mutations and four reported mutations involved two unique frameshift (Tyr655ValfsTer18 in *BRCA1* and c.1286_1287insA in *BRCA2*), two splicing (c.5074_5074+4delGGTAT in *BRCA1* and Arg2336Alafs [7006delC] in *BRCA2*) and three nonsense (Tyr130Ter in *BRCA1*, Arg2494Ter, and Glu1113Ter in *BRCA2*) mutations. All others are missense mutations. Further segregation and functional studies are needed to identify the pathogenicity of these variants (Table 2).

DISCUSSION

We confirmed 20 different *BRCA1/2* mutations in 25 (21.9%) of 114 breast cancer patients with a family history of breast cancer using PCR-based 48.48 Access Array microfluidic technology (Fluidigm). Six have been reported as disease-causing mutations in public databases (BIC and HGMD). Two *BRCA1* and four *BRCA2* mutations in eight patients were novel mutations (not reported in any variant database or previous study).

Two mutations (Tyr130Ter in *BRCA1* and Arg2494Ter in *BRCA2*) that we identified are frequently reported in Asians. Tyr130Ter (stop gained) is the most common *BRCA1* gene mutation and is frequently observed in Koreans, Japanese, and Chinese.[20,21] Arg2494Ter is the most common *BRCA2* mutation found in Koreans including in the United States (Los Angeles and Stanford).[20] Interestingly, seven mutations in this study were in the same position as mutations found in previous studies, but were different types. Tyr655ValfsTer18 in *BRCA1* is a duplication frameshift found in Chinese patients;[22] we also found a deletion frameshift (Lys654SerfsTer47) in Chinese and Korean patientss.[23,24] Various intervening sequences were found, including c.5074 +1G>A or T and +3A>G and c.5075 −1G>T or −2A>T including c.5074_5074+4delGGTAT in *BRCA1*.[23,25-27] Val409 in *BRCA1* replaced Ter in Japan and Leu in this study.[25] In *BRCA2* in this study, Ser1632 Asn, Tyr1710Asp, Arg2336Alafs (7006delC), and Gln3026Glu were found as insertion, deletion, missense or deletion, and nonsense mutations in other studies.[23,25] These mutations indicate that some sites in the gene are frequently mutated.

In our study, the *BRCA1/2* mutation frequency (21.9%) was similar to other reports (19.6% and 20.6%) of familial breast cancer patients in the Korean population.[12,14] These mutation frequencies were much higher in familial breast cancer patients than in patients with no family history of breast or ovarian cancer (8.6%)[20] or with sporadic breast cancer (3.1% and 10%)[13,28] in Koreans. The prevalence of *BRCA1/2* mutation among breast cancer patients varies in Western (1.8-3.6%)[11] and Asian (0.8-4.4%) countries.[24,25] The rate of deleterious mutations in *BRCA1* and *BRCA2* has been reported to be 20-40% in familial breast cancer patients.[29,30] Among the 20 mutations found in our study, two were frameshift, two were splicing and three were nonsense mutations. These three types of mutations result in a completely different or often nonfunctional protein products. Therefore, these were considered possible pathogenic mutations.

The differences in reported results may be due to the different populations studied, techniques used (e.g., direct sequencing, next-generation sequencing [NGS], or targeting only several mutations and not whole exons), and / or patient selection criteria. As large insertions/deletions could not be detected by the Access Array-based NGS used in this study, the mutation rate in our study population might be higher, although the rate of large insertions/deletions is low. Therefore, further studies should examine more cases and mutations of entire exons. Further segregation and functional studies are needed to clarify the pathogenicity of these variants.

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CONFLICT OF INTEREST STATEMENT

None declared.

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