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Original Article

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BRCA1/2 mutations, including large genomic rearrangements, among unselected ovarian cancer patients in Korea

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ABSTRACT

Objective: We performed small-scale mutation and large genomic rearrangement (LGR) analysis of *BRCA1/2* in ovarian cancer patients to determine the prevalence and the characteristics of the mutations.
Methods: All ovarian cancer patients who visited a single institution between September 2015 and April 2017 were included. Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA), and long-range polymerase chain reaction (PCR) were performed to comprehensively study *BRCA1/2*. The genetic risk models BRCAPRO, Myriad, and BOADICEA were used to evaluate the mutation analysis.
Results: In total, 131 patients were enrolled. Of the 131 patients, Sanger sequencing identified 16 different *BRCA1/2* small-scale mutations in 20 patients (15.3%). Two novel nonsense mutations were detected in 2 patients with a serous borderline tumor and a large-cell neuroendocrine carcinoma. MLPA analysis of *BRCA1/2* in Sanger-negative patients revealed 2 LGRs. The LGRs accounted for 14.3% of all identified *BRCA1* mutations, and the prevalence of LGRs identified in this study was 1.8% in 111 Sanger-negative patients. The genetic risk models showed statistically significant differences between mutation carriers and non-carriers. The 2 patients with LGRs had at least one blood relative with breast or ovarian cancer.
Conclusion: Twenty-two (16.8%) of the unselected ovarian cancer patients had *BRCA1/2* mutations that were detected through comprehensive *BRCA1/2* genetic testing. Ovarian cancer patients with Sanger-negative results should be considered for LGR detection if they have one blood relative with breast or ovarian cancer. The detection of more *BRCA1/2* mutations in patients is important for efforts to provide targeted therapy to ovarian cancer patients.

Keywords: Mutation; Genes; Neoplasms; Ovary; Korea

INTRODUCTION

Ovarian cancer, which is the most fatal gynecologic malignancy in the world, is a heterogeneous disease with multiple histologic subtypes. It is the fourth most common

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Author Contributions

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cancer in females aged 0–34, and its prevalence has been increasing in Korea [1]. The majority (90%) of ovarian cancers are epithelial ovarian cancers [2]. The most common histologic subtype of epithelial ovarian cancer is high-grade serous ovarian cancer, accounting for about 70% of cases, a majority of which are diagnosed at an advanced stage [3].

High-penetrance ovarian cancer susceptibility genes *BRCA1* and *BRCA2* are tumor suppressor genes located at 17q21.31 and 13q13.1, respectively. In a recent prospective cohort study, the cumulative risk of ovarian cancer by age 80 was 44% for *BRCA1* mutation carriers and 17% for *BRCA2* mutation carriers [4]. In May 2013, Angelina Jolie, an actress from the United States, publicized her story of having a *BRCA1* mutation and undergoing risk-reducing surgery. After her announcement, referrals to genetic services and risk-reducing surgeries increased, the so-called "Angelina effect." Moreover, In Korea, the National Health Insurance system promoted a strategy of *BRCA1/2* testing coverage for epithelial ovarian cancer patients in May 2012. Both the "Angelina effect" and Health insurance coverage are thought to be behind the gradual increase in *BRCA* testing in Korea [5].

The majority of *BRCA1/2* mutations are small-scale mutations (point mutations, small deletions, or insertions), resulting in protein truncation, disruption of messenger RNA processing, or amino acid substitutions that have a significant impact on protein function. Such mutations occur throughout the whole coding sequence or at the splice junctions of both genes. These mutations are readily detected by standard Sanger sequencing methods using polymerase chain reaction (PCR)-amplified gene segments [6]. Another mechanism of *BRCA1* and *BRCA2* inactivation are large genomic rearrangements (LGRs), which are responsible for a variable but significant proportion of *BRCA1/2* mutations [7]. Because Sanger sequencing is incapable of detecting LGRs, other techniques, such as multiplex ligation-dependent probe amplification (MLPA) or next-generation sequencing, are required. MLPA is a semi-quantitative technique that is the most commonly used method for detecting LGRs in *BRCA1/2*. A high prevalence of LGRs in *BRCA1* or *BRCA2* has been demonstrated in several populations, including the Dutch, Northern Italian, Danish, and Portuguese [8-11]. In contrast, studies in some populations, including the French-Canadian and Sri Lankan, found no LGR mutations in *BRCA1/2*, suggesting that LGRs are probably very rare in such populations [12,13]. In populations with rare LGRs, a cost-effective screening strategy for LGR detection is necessary to identify all patients that may benefit from targeted therapies, such as a poly ADP-ribose polymerase inhibitor.

According to a recent study in Korea, 24.6% of 232 epithelial ovarian patients had small-scale mutations of *BRCA1/2* that were detected using Sanger sequencing [14]. A few LGRs in primary breast cancer patients were also reported in Korea [15-17], but the prevalence of LGRs in primary ovarian cancer patients has not yet been reported in Korea. Additionally, most previous *BRCA1/2* studies in ovarian cancer have been focused on epithelial subtypes, but *BRCA1/2* mutations in various other ovarian cancer histologic subtypes have also been reported [18,19].

Therefore, we performed small-scale mutation and LGR analysis without selection in multiple ovarian cancer subtypes. We also evaluated the usefulness of several mutation carrier prediction algorithms for predicting *BRCA1/2* mutations, including LGRs. Here, we report the results of a comprehensive *BRCA1/2* genetic study that determined the prevalence and the characteristics of mutations among unselected ovarian cancer patients.

MATERIALS AND METHODS

1. Study population

All ovarian, primary peritoneal, primary fallopian tube cancer patients who visited the Department of Obstetrics and Gynecology at Dongsan Medical Center of Keimyung University for genetic testing between September 2015 and April 2017 were included in this study. The family histories, medical record, and tumor pathology of the probands and their family members were detailed through genetic counseling and/or review of the patient's medical record. All participants provided informed consent, and this study was approved by the Institutional Review Board (IRB)/ Ethics Committee of Dongsan Medical Center (IRB No. 2016-05-002).

2. Sanger sequencing, MLPA, and long-range PCR

Sanger sequencing was performed in all ovarian cancer patients to detect small-scale mutations. Genomic DNA was isolated from the peripheral blood leukocytes using the QIAmp DNA Mini Kit (Qiagen, Hamburg, Germany). Sanger sequencing was performed on a 3500xL DNA Analyzer with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and analyzed using Sequencer 5.0 software (Gene Codes Corporation, Ann Arbor, MI, USA). Exon numbering and DNA sequence variant descriptions of *BRCA1/2* were based on NM_007294.3 (NG_005905.2) and NM_000059.3 (NG_012772.3) as reference sequences, obtained from the NCBI RefSeq database (https://www.ncbi.nlm.nih.gov/refseq/rsg/). All *BRCA1/2* variants were categorized into pathogenic, variants of uncertain significance (VUS), or neutral. Pathogenic variants were defined as previously reported pathogenic variants and novel nonsense or frameshift variants that resulted in protein truncation. Neutral variants were those that were clearly not pathogenic or were unlikely to be pathogenic. Other variants were classified as VUS.

MLPA was performed on Sanger sequencing-negative patients. MLPA probe mixes P002 and P045 were used for the screening of LGRs in *BRCA1* and *BRCA2*, respectively, and P087 and P077 were used for confirmation, according to the manufacturer's recommendations (MRC-Holland, Amsterdam, The Netherlands). MLPA was performed as previously described [15]. Genemarker v1.91 software (Softgenetics, State College, PA, USA) was used for fragment analysis. After normalization of raw data, a deletion or duplication was identified when the peak height ratio was below 0.70 or more than 1.40, respectively. We also used Sanger sequencing of the probe binding and ligation sites to detect any variants that may have led to a false positive result [6,20].

To characterize the LGRs detected in this study, long-range PCR was performed using primers Int20-3′ F 5′-CCTGGGAGAACCCCAGAGT-3′ and Int20-3′ R 5′-CTGGTCCTGGAGGAGGAGTT-3′ and an Expand Long Range dNTPack (Roche Diagnostics GmbH, Mannheim, Germany). The PCR cycling conditions were as follows: an initial denaturation at 92°C for 2 minutes; 10 cycles at 92°C for 10 seconds, 60°C for 15 seconds, and 68°C for 15 minutes; 25 cycles at 92°C for 10 seconds, 60°C for 15 seconds, and 68°C for 15 minutes (increase of 20 seconds per cycle); and final extension at 68°C for 15 minutes.

To determine the exact breakpoint of LGR, the target band was cut from the agarose gel and was employed to purify the target fragment using SolGent UB solution (SolGent, Daejeon, Korea). The product was sequenced with a series of additional primers to successfully narrow down the breakpoint regions. The primer that eventually allowed for characterization of the exon 21–23 deletion breakpoint was Internal1 F 5′-GAGGTCGAGTCTCTGTTGCC-3′.

The breakpoint of LGR was described according to the Human Genome Variation Society (HGVS) nomenclature criteria (http://varnomen.hgvs.org/recommendations/). To explore the mechanism underpinning LGR, L78833.1 was used as a reference sequence to search for the location of *Alu* sequences.

3. Mutation carrier prediction algorithms for *BRCA1/2*

We used the genetic risk models BRCAPRO, Myriad, and BOADICEA to compute a probability or a score for the likelihood of carrying a *BRCA1/2* mutation.

BRCAPRO is a statistical model that uses Mendelian inheritance and Bayesian analysis based on all available data, including current age/age at death, age of diagnosis, ethnicity, personal/familial history of cancer, cancer markers, and genetic test results [21]. BRCAPRO scores were calculated using the latest version of the software program, CancerGene 6.0 (available at http://www4.utsouthwestern.edu/breasthealth/cagene/).

The Myriad *BRCA1* and *BRCA2* prevalence tables provide the probability of detecting *BRCA1/2* mutations and are based on observations of deleterious mutations by Myriad Genetic Laboratories through its clinical testing service [22]. We used the latest version of the table, which was based on 162,914 tests in individuals without an Ashkenazi ancestry (available at https://new.myriadpro.com/products/bracanalysis-overview/#1479845138516-ee8a82d1-427c).

BOADICEA is a computer program that is used to calculate the risk of breast or ovarian cancer in women and the probability of being a *BRCA1/2* mutation carrier based on family history [23]. BOADICEA scores were calculated using the latest version of software, BOADICEA Web Application v3 (available at https://pluto.srl.cam.ac.uk/cgi-bin/bd3/v3/bd.cgi).

4. Statistical analyses

Categorical variables were compared using a χ^2 test or Fisher's exact test. Continuous variables were compared using the Mann-Whitney U test. To determine the performance of the mutation carrier prediction algorithms, receiver operating characteristics (ROC) curves were estimated, and the area under the ROC curve and 95% confidence interval (CI) were calculated. SPSS version 20 (IBM Corp., Armonk, NY, USA) was used. A p value of <0.05 was considered statistically significant.

RESULTS

In total, 131 patients were enrolled. All patients were of Korean ethnicity, and the mean age at diagnosis was 52 years. Of the 131 patients, 126 had ovarian cancer and 5 had primary peritoneal or fallopian tube cancer. Five patients had a personal history of breast cancer, and 15 patients had close blood relatives (including first-, second-, or third-degree relatives) with *BRCA*-associated cancer (breast, ovarian, primary peritoneal, fallopian tube, pancreatic, or prostate cancer). Histology of the tumors revealed that 87 patients (66.4%) had serous type and 44 (33.6%) patients had a non-serous type. Sixty-nine patients (52.7%) had advanced-stage (III, IV) cancer. Ninety-nine patients (75.6%) had a high-grade cancer (**Table 1**).

Of the 131 patients, Sanger sequencing identified 10 different *BRCA1* small-scale mutations in 12 patients (9.2%) and 6 *BRCA2* small-scale mutations in 8 patients (6.1%). Of the 16 *BRCA1/2* small-scale mutations, 2 *BRCA1* mutations (c.3655G>T and c.4253delT) were novel. One

Table 1. Baseline characteristics of the patients (n=131)

Characteristic	Value
Age at diagnosis (yr)	52 (20–74)
Type of cancer	
Ovarian	126 (96.2)
Primary peritoneal	2 (1.5)
Primary fallopian tube	3 (2.3)
Personal history of breast cancer	5 (3.8)
Family history (1st– 3rd degree relatives) of *BRCA*-associated cancer*	15 (11.5)
Tumor histology	
Serous	87 (66.4)
Clear cell	18 (13.7)
Mucinous	9 (6.9)
Endometrioid	9 (6.9)
Seromucinous	2 (1.5)
Serous borderline tumor	1 (0.8)
Squamous cell	2 (1.5)
Sertoli-Leydig cell	1 (0.8)
Carcinosarcoma	1 (0.8)
Large cell neuroendocrine	1 (0.8)
Stage	
I–II	62 (47.3)
III–IV	69 (52.7)
Tumor grade	
1–2	32 (24.4)
3	99 (75.6)

Data are described as the mean (range) or number (%).
*_BRCA_-associated cancers include breast, ovarian, primary peritoneal, fallopian tube, pancreatic, and prostate.

BRCA1 mutation (c.5080G>T, n=3) and 2 *BRCA2* mutations (c.1399A>T, n=2; c.7480C>T, n=2) were recurrent (**Table 2**). One *BRCA2* mutation (c.3096_3110delinsT) was found in a patient with primary fallopian tube cancer.

MLPA analysis of *BRCA1/2* in Sanger-negative patients revealed 2 LGRs: a deletion of *BRCA1* exons 1–2 in patient A (**Fig. 1A**) and a deletion of *BRCA1* exons 21–23 in patient B (**Fig. 1B**).

Table 2. Deleterious *BRCA1/2* gene mutations found in this study

Gene	Exon/intron	Nucleotide change*	Protein change*	Mutation type	NCBI SNP ID number	No.
BRCA1	Exon6	c.390C>A	p.Tyr130Ter	Nonsense	rs80356888	1
	Exon10	c.1354delG	p.Val452Ter	Nonsense	rs886039946	1
		c.1831delC	p.Leu611Ter	Nonsense	rs397508913	1
		c.3296delC	p.Pro1099Leufs	Frameshift	rs80357815	1
		c.3627dupA	p.Glu1210Argfs	Frameshift	rs80357729	1
		c.3655G>T	p.Glu1219Ter	Nonsense	Novel	1
	Exon12	c.4253delT	p.Leu1418Ter	Nonsense	Novel	1
		c.4327C>T	p.Arg1443Ter	Nonsense	rs41293455	1
	Exon17	c.5080G>T	p.Glu1694Ter	Nonsense	rs80356896	3
	Exon23	c.5496_5506delinsA	p.Val1833Serfs	Frameshift	rs273902775	
	Exon1–2	c.(?_-232)_(c.80+1_81-1)del		LGR	-	1
	Exon21–23	c.5332+558_*6809del		LGR		1
BRCA2	Exon10	c.1399A>T	p.Lys467Ter	Nonsense	rs80358427	2
	Exon11	c.3096_3110delinsT†	p.Lys1032Asnfs	Frameshift	rs397507655	1
		c.6553delG	p.Ala2185Leufs	Frameshift	rs80359603	1
		c.7480C>T	p.Arg2494Ter	Nonsense	rs80358972	2
	Exon23	c.9117G>A	p.Pro3039=	Splice site	rs28897756	1
	Exon24	c.9253delA	p.Thr3085Glnfs	Frameshift	rs397508041	1

NCBI, National Center for Biotechnology Information; SNP, single nucleotide polymorphism; HGVS, Human Genome Variation Society.
*All variants were described according to the recommended HGVS nomenclature; †This variant was found in a patient with primary fallopian tube cancer.

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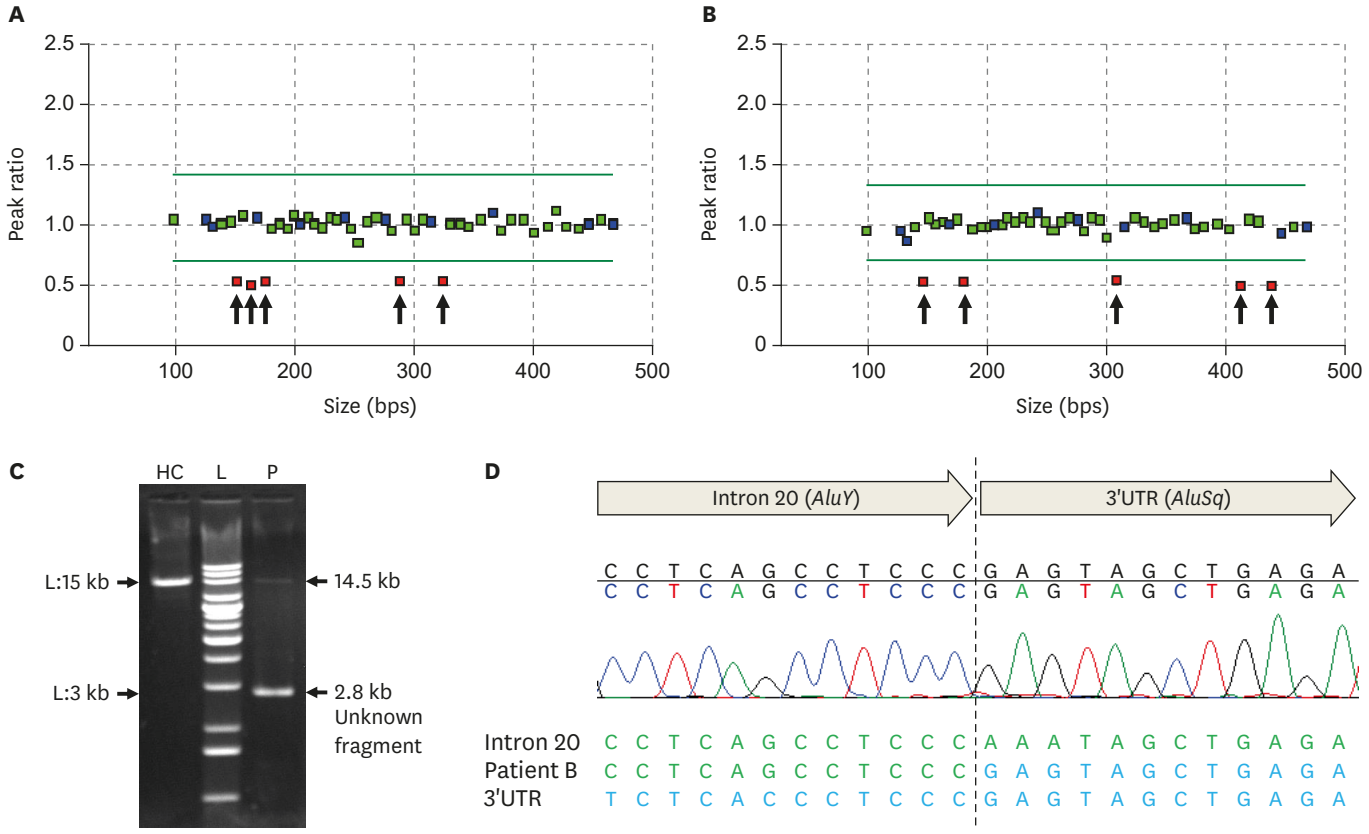

Fig. 1. MLPA analysis of patient A (A) and B (B). The red squares indicate the deleted *BRCA1* probes. (C) Long-range PCR results for patient B showing an 11.7 kb-sized deletion of the *BRCA1* gene. (D) Sanger sequencing of the abnormal band revealed the LGR breakpoint: NG_005905.2:g.167479_179115del11637. This LGR fuses the *AluY* sequence with the *AluSq* sequence.
MLPA, multiplex ligation-dependent probe amplification; PCR, polymerase chain reaction; LGR, large genomic rearrangement; HC, healthy control; L, DNA extension ladder; P, patient B; UTR, untranslated region.

The long-range PCR in patient B revealed a 2.8-kb-sized unknown fragment with a 14.5-kb-sized normal fragment, showing an approximately 11.7-kb-sized deletion of the *BRCA1* gene (**Fig. 1C**). Finally, the subsequent Sanger sequencing of this unknown fragment revealed the exact breakpoint of the *BRCA1* LGR in patient B. The patient had an 11,637-bp-sized deletion that ranged from intron 20 to the 3′ untranslated region (UTR). The LGR in patient B was described as NG_005905.2(NM_007294.3):c.5332+558_*6809del. Because the deletion breakpoint of *BRCA1* LGR in patient A was uncharacterized, the description of the LGR was NG_005905.2(NM_007294.3):c.(?_-232)_(c.80+1_81-1)del according to the Human Genome Variation Society nomenclature criteria. This LGR fuses the *AluY* sequence in intron 20 with the *AluSq* sequence of the 3′ UTR (**Fig. 1D**). In total, including the two *BRCA1* LGR cases, 22 (16.8%) of the 131 enrolled patients had *BRCA1/2* mutations. The LGRs consisted of 14.3% of all identified *BRCA1* mutations, and the prevalence of LGRs identified in this study was 1.8% in 111 Sanger-negative patients and 1.5% in all enrolled patients (**Table 2**).

The pedigrees of the 2 patients with LGRs are shown in **Fig. 2**. Patient A, who was diagnosed with stage III high-grade serous ovarian cancer at age 65, had one first-degree blood relative with ovarian cancer (**Fig. 2A**). Patient B, who was diagnosed with stage III high-grade serous ovarian cancer at age 45, had one second-degree blood relative with breast cancer and one first-degree blood relative with stomach cancer (**Fig. 2B**).

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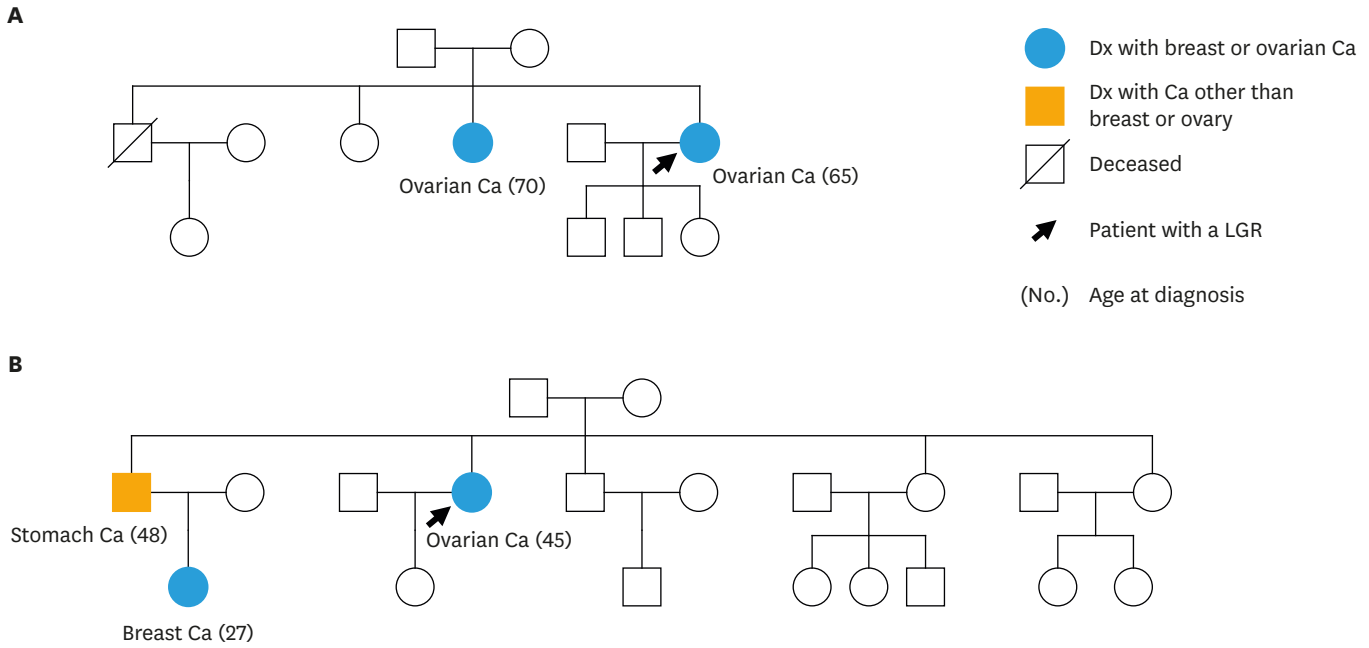

Fig. 2. Pedigree of the 2 patients with LGR mutations in this study. (A) Family pedigree of patient A with a deletion of *BRCA1* exons 1–2. (B) Family pedigree of patient B with a deletion of *BRCA1* exons 21–23.
LGR, large genomic rearrangement; Ca, cancer; Dx, diagnosed.

The characteristics of the patients according to *BRCA1/2* mutation status are shown in **Table 3**. Patients with *BRCA1/2* mutations were more likely to have a personal history of breast cancer (p=0.033) and a family history (including third-degree blood relatives) of *BRCA*-associated cancer (p<0.001). Among the 22 patients with *BRCA1/2* mutations, 18 (81.8%) had serous histologic type and two patients (9.1%) had endometrioid histologic type. Two patients (9.0%) with *BRCA1/2* mutations had serous borderline tumor and large-cell neuroendocrine carcinoma (LCNC) types. The BRCAPRO, Myriad, and BOADICEA scores were significantly higher among patients with *BRCA1/2* mutations (p=0.006, p<0.001, and p=0.040, respectively).

The estimated ROC curves for all patients are shown in **Supplementary Fig. 1**. The areas under the ROC curves for predicting the probability of BRCA1/2 mutations were 0.685 (95% CI=0.538–0.831), 0.660 (95% CI=0.518–0.803), and 0.639 (95% CI=0.486–0.793) for the BRCAPRO, Myriad, and BOADICEA scores, respectively. The suggested cutoff point of BRCAPRO by the closest value to the left upper corner was 3.04%, and the sensitivity and specificity values at the cutoff points were 0.636 and 0.789, respectively. The suggested cutoff point of Myriad by the closest value to the left upper corner was 9.9%, and the sensitivity and specificity values at the cutoff point were 0.364 and 0.954, respectively. The suggested cutoff point of BOADICEA by the closest value to the left upper corner was 3.5%, and the sensitivity and specificity values at the cutoff point were 0.545 and 0.807, respectively. Using a 10% cutoff, the traditional cutoff for offering a *BRCA1/2* genetic testing, 16 mutations (72.7%) in BRCAPRO, 14 mutations (63.6%) in Myriad, and 16 mutations (72.7%) in BOADICEA would have been missed. Even if we had used each suggested cutoff point from the ROC curves, all of which were lower than 10%, the total number of missed mutations would be 8 (36.4%) using BRCAPRO, 14 (63.6%) using Myriad, and 10 (45.5%) using BOADICEA.

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Table 3. Characteristics of the patients according to *BRCA1/2* mutation status

Characteristic	*BRCA* mutation positive (n=22)	*BRCA* mutation negative (n=109)	p value
Age at diagnosis (yr)	57.1 (41–73)	51.5 (20–74)	
Type of cancer			
Ovarian	21 (95.5)	105 (96.3)	
Primary peritoneal	0	2 (1.8)	
Primary fallopian tube	1 (4.5)	2 (1.8)	
Personal history of breast cancer	3 (13.6)	2 (1.8)	0.033
Family history (1st–3rd degree relatives) of *BRCA*-associated cancer	9 (40.9)	6 (5.5)	<0.001
Tumor histology			
Serous	18 (81.8)	69 (63.3)	
Clear cell	0	18 (16.5)	
Mucinous	0	9 (8.3)	
Endometrioid	2 (9.1)	7 (6.4)	
Seromucinous	0	2 (1.8)	
Serous borderline tumor	1 (4.5)	0	
Squamous cell	0	2 (1.8)	
Sertoli-Leydig cell	0	1 (0.9)	
Carcinosarcoma	0	1 (0.9)	
Large cell neuroendocrine	1 (4.5)	0	
Stage			0.509
I–II	9 (40.9)	53 (48.7)	
III–IV	13 (59.1)	56 (51.3)	
Tumor grade			0.197
1–2	3 (13.6)	29 (26.6)	
3	19 (86.4)	80 (73.4)	
BRCAPRO score	0.113 (0.011–0.802)	0.032 (0.003–0.300)	0.006
Myriad score	0.105 (0.077–0.263)	0.080 (0.077–0.147)	<0.001
BOADICEA score	0.081 (0.005–0.511)	0.029 (0.002–0.291)	0.040

Data are described as the mean (range) or number (%).

DISCUSSION

Of the 131 unselected Korean ovarian (including primary peritoneal or fallopian tube) cancer patients evaluated in this study, *BRCA1/2* mutations, including the two LGRs, were detected in 22 (16.8%). This is a lower mutation prevalence than found by a previous study on Korean patients (24.6%) [14], and it may have resulted from our unselective inclusion of all ovarian cancer patients. The BRCAPRO, Myriad, and BOADICEA scores were significantly higher for patients with *BRCA1/2* mutations.

BRCAPRO, Myriad, and BOADICEA are the most widely used models for estimating the likelihood that a *BRCA1/2* mutation is present based on family history. BRCAPRO and Myriad models incorporate the effects of breast and ovarian cancer. The BOADICEA model incorporates information on breast, ovarian, pancreatic, and prostate cancer. The BRCAPRO, Myriad, and BOADICEA scores were significantly higher in *BRCA1/2* mutation carriers compared to non-carriers; however, many patients classified as low risk by these models were much more likely than predicted to have *BRCA1/2* mutations. These findings support a universal testing strategy for all ovarian cancer patients regardless of family history as assessment of family history by validated prediction models cannot effectively target testing to a high-risk ovarian cancer patient population [24].

Interestingly, 2 novel nonsense mutations were detected in 2 patients with a serous borderline tumor and LCNC. Ovarian borderline tumors, otherwise known as ovarian tumors of low malignant potential (LMP), are a histologic subtype of epithelial ovarian cancer.

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According to a recent report, aggressive tumor characteristics were suggested as a common feature in ovarian LMP tumors with *BRCA1/2* mutations [25]. In this study, the patient had a stage III serous borderline tumor and was diagnosed with hepatic metastasis at age 47. This patient died from hepatic failure. Therefore *BRCA1/2* testing should be considered in patients with ovarian LMP tumors who exhibit aggressive tumor characteristics. LCNC, which is an aggressive cancer with a tendency to present at advanced stages, has no standard treatment. LCNC is a rare cancer, and only a few cases have been reported in the literature [26]. Only one previous study reported a *BRCA2* germline mutation in a patient with LCNC of the ovary [19], and, to the best of our knowledge, this is the first report of a *BRCA1* germline mutation carrier with LCNC of the ovary. Therefore, even ovarian cancer patients with non-epithelial histological subtypes, especially LCNC, should be considered for *BRCA1/2* genetic testing.

The LGRs consisted of 9.1% of all detected *BRCA1/2* mutations, and the prevalence of LGRs identified in this study was 1.8% in 111 Sanger-negative patients and 1.5% in all enrolled patients. The major mechanisms of LGRs found in *BRCA1/2* are *Alu*-mediated unequal homologous recombination and non-homologous events, such as *Alu*/non-*Alu* or non-*Alu*/non-*Alu*. The breakpoint analysis of LGR in patient B revealed a fusion between the *AluY* sequence in intron 20 and the *AluSq* sequence of the 3′ UTR, which is suggestive of an unequal homologous recombination event between these two *Alu* sequences that share 84% homology. Until recently, only *BRCA1* LGR cases had been reported in Korea [15-17,27], and our two LGR cases also involved *BRCA1*. As no LGRs in *BRCA2* have been identified, these mutations must be rare in our population. Of the reported *BRCA1/2* LGR cases in other populations, *BRCA1* LGRs were in the majority [10,28]. *BRCA1* contains more *Alu* sequences than *BRCA2*, which may explain why more LGRs have been reported in *BRCA1* [28].

In populations with a low *BRCA1/2* LGR prevalence, LGR screening is rarely performed in routine genetic laboratories. Therefore, patients with *BRCA1/2* LGR mutations who may benefit from targeted therapies would be missed by routine genetic testing. For such populations, an effective screening strategy for *BRCA1/2* LGR detection is necessary to enable a more efficient and lower-cost mutational screening approach. In Korea, only three ovarian patients with *BRCA1* LGRs, including the 2 patients in this study, have been reported (**Table 4**). All three patients had high-grade serous ovarian cancer, which was diagnosed at ages 45, 35, and 65 years. Each patient had at least one blood relative with breast or ovarian cancer, and this finding is similar to that of a previous large-scale study of *BRCA1/2* LGRs [29]. Additionally, in our study, most BRCAPRO, Myriad, and BOADICEA scores were higher in patients carrying *BRCA1/2* mutations. These results partially support a previous study that showed a positive correlation between the proportion of mutations due to a LGR and the BRCAPRO score. Therefore, Sanger-negative ovarian cancer patients should be considered for LGR testing if they have one blood relative with breast or ovarian cancer. Additionally, an above-average score on the BRCAPRO, Myriad, or BOADICEA assessments could also be an indication for LGR testing.

Table 4. Characteristics of ovarian cancer patients with *BRCA1* LGRs reported in Korea

Exon rearrangement	Age at Dx (yr)	FHx of *BRCA*-associated cancer	Tumor histology	Tumor grade	Stage	BRCAPRO score	Myriad score	BOADICEA score	Reference
Deletion of exon 1–2	45	1 BC	serous	3	III	0.199	0.143	0.164	This study
Duplication of exon 4–6	35	1 BC, 1OC	serous	3	III	0.730	0.344	0.398	[17]
Deletions of exon 21–23	65	1OC	serous	3	III	0.089	0.147	0.024	This study

LGR, large genomic rearrangement; Dx, diagnosis; FHx, family history; BC, breast cancer; OC, ovarian cancer.

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This study had several limitations. First, as this was a single-institution study performed over 18 months, the number of non-epithelial ovarian cancer or ovarian LMP tumors was small (n=6). Additionally, more detailed data on the characteristics of *BRCA1/2* LGR mutations in Korea could be gathered through larger studies. Second, some family histories were taken from medical records, which may have introduced bias. Despite these limitations, the current study identified 2 mutations in patients with a serous borderline tumor and LCNC. We also found two *BRCA1* LGRs in patients with a family history of *BRCA*-associated cancer. These mutations accounted for 9.1% of all detected *BRCA1/2* mutations and were found in 1.8% of Sanger-negative patients. A larger multicenter study would improve the statistical significance of research in this area.

In conclusion, 16.8% of unselected ovarian cancer patients had *BRCA1/2* mutations detected through a comprehensive *BRCA1/2* genetic study. We identified two rare cases of *BRCA1* mutations in patients with an ovarian LMP tumor and LCNC. Therefore, patients with ovarian LMP tumors with aggressive characteristics and LCNC of the ovary should be considered for *BRCA1/2* genetic testing. We also found 2 cases with *BRCA1* LGRs. Based on these results, ovarian cancer patients who are Sanger-negative should be considered for LGR detection if they have one blood relative with breast or ovarian cancer. Additionally, an above-average score on the BRCAPRO, Myriad, or BOADICEA assessments may also indicate the need for LGR testing in Sanger-negative patients. A cost-efficient mutation testing strategy will detect more *BRCA1/2* mutations in ovarian cancer patients in populations with a low LGR prevalence. This is essential for the ability to provide targeted therapies to and thereby increase the survival rate of patients with ovarian cancer.

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SUPPLEMENTARY MATERIAL

Supplementary Fig. 1

ROC curve for the BRCAPRO, Myriad, and BOADICEA scores. The areas under the ROC curves were 0.685 (95% CI=0.538–0.831), 0.660 (95% CI=0.518–0.803), and 0.639 (95% CI=0.486–0.793) for the BRCAPRO (blue line), Myriad (green line), and BOADICEA (yellow line) scores, respectively. ROC, receiver operating characteristics; CI, confidence interval.

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