Prevalence and clinical characterization of BRCA1 and BRCA2 mutations in Korean patients with epithelial ovarian cancer

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Abstract
This study was performed to investigate the prevalence, clinical characteristics, and treatment response according to BRCA1 and BRCA2 (BRCA) mutations in Korean patients with epithelial ovarian cancer (EOC). Two-hundred and ninety-eight Korean women diagnosed with high-grade serous and/or endometrioid EOC from 2010 to 2015 were tested for germline and 86 specimens for somatic BRCA mutations, regardless of the family history. Clinical characteristics including survival outcomes were compared in patients with and without BRCA mutations (NCT02963688). A total of 43 different germline BRCA mutations were identified in 78 patients among 298 patients (26.2%). Somatic BRCA mutations were identified in 11 (12.8%) patients among patients without germline BRCA mutations. Haplotype analysis demonstrated no founder mutations in our Korean patient cohort. Insignificant differences in age at diagnosis, primary site, and residual disease after surgery were observed between patients with and without BRCA mutations. In multivariate analysis for overall survival (OS), the presence of BRCA mutation was significantly associated with OS (P = .049) in addition to platinum sensitivity (P < .001), indicating it is an independent prognostic factor for survival regardless of platinum sensitivity to first-line chemotherapy. In addition, a higher response rate to subsequent chemotherapy after recurrence was observed in EOC patients with BRCA mutations resulting in better OS. In the current study, the prevalence of BRCA mutations in Korean patients with EOC was higher than previously reported in other ethnic groups. We demonstrated characteristics and treatment response in Korean EOC patients with BRCA mutations. These findings may provide valuable information to be considered in future clinical trials including Asian patients.

KEYWORDS
BRCA1 genes, BRCA2 genes, germline mutation, ovarian neoplasm, overall survival
Epithelial ovarian cancer (EOC) is the second leading cause of death related to gynecologic cancers and the eighth most common leading cause of death from cancer in women worldwide. The standard therapy for EOC consists of surgical cytoreduction and adjuvant treatment with taxane and platinum-based chemotherapeutic agents. Despite a positive response to taxane and platinum-based chemotherapeutic agent in patients with advanced EOC, most patients experience relapse. Repeated chemotherapy in recurrent EOC patients diminishes effectiveness and leads to cumulative increased toxicity. Recent trends in treatment for gynecological cancer fields include incorporation of molecular target therapy in chemotherapy. As a result, bevacizumab (humanized monoclonal antibody targeting vascular endothelial growth factor) and poly adenosine diphosphate-ribose polymerase inhibitor (PARPi) maintenance have shown clinical benefits in EOC patients.

The significant activity of PARPi in BRCA1 and BRCA2 (BRCA) mutation carriers also drew attention in BRCA testing early in the management of EOC. In the SOLO-1 study, a phase 3 randomized controlled trial (RCT) in advanced EOC patients with germline BRCA mutation (gBRCAm) or somatic BRCA mutation (sBRCAm), olaparib maintenance therapy showed significantly prolonged progression-free survival (PFS). Velaparib and niraparib maintenance therapy in newly diagnosed EOC patients showed survival benefit regardless of the presence of BRCA mutations (BRCAm). but survival benefit for disease progression was clearer in the subgroup with BRCAm. PARPi (olaparib, rucaparib, and niraparib) showed its efficacy in EOC patients for the maintenance therapy of relapsed platinum-sensitive EOC. In addition, PAPRi, in single or multiple combinations including anti-angiogenic agents and immune check-point inhibitors, is under extensive investigation in clinical trials in patients with first-line setting of EOC as well as in recurrent settings. However, there is still a need for investigation of predictive molecular biomarkers and the best combination for PARPi in the treatment of EOC patients.

Approximate frequency of gBRCAm in EOC widely vary from 5% to 30%, and less frequent occurrence of sBRCAm (2%-8%) has been reported in patients with EOC. EOC patients with gBRCAm were reported to have better survival outcome compared to patients without gBRCAm, and the mechanism underlying this benefit was hypothesized as a high response rate to platinum agents, particularly in patients with high-grade serous type. The reason is that patients with gBRCAm are impaired in their ability to repair double-stranded DNA breaks through homologous recombination (HR). Despite the discovery of BRCA more than 20 years ago, almost all the available data are from women in the United States, Europe, and Australia, and there have only been a few studies, mostly with small sample size, in Asian populations. Recently, many international clinical trials have been recruiting Asian patients and several clinical trials have exhibited different outcomes between European and Asian EOC patients, for example pazopanib maintenance therapy and dose dense weekly paclitaxel plus tri-weekly carboplatin chemotherapy in first-line treatment in ovarian cancer. BRCAm has recently become known as a prognostic factor in EOC, therefore it is necessary to understand more fully its prevalence and clinical characteristics, including the treatment response of EOC patients with BRCAm in Asian populations, through a larger scale study and comprehensive analysis of its clinical characterization.

Thus, we evaluated the clinical characterization and treatment response of EOC patients with the BRCA mutation in 298 Korean patients with high-grade serous and/or endometrioid EOC.

2 | METHODS

2.1 | Patients and study design

This study is a result of retrospectively analyzed subgroup data based on the Korean Gynecologic Oncology Group (KGOG) 3019 study, which was a prospective, multicenter observational study conducted in Korea from 2010 to 2015 to identify environmental and personal risk factors for EOC. According to the KGOG 3019 protocol, we collected blood, urine, and tumor samples from patients simultaneously at operation room. Patients were included after histological diagnosis of EOC and were not selected for age or family history. Patient interviews were done in hospital with detailed questionnaires asking about known and suspected ovarian cancer risk factors. A flowchart of the study is shown in Figure 1. We extracted 565 patients from KGOG 3019 data for this study that fitted our inclusion criteria: eligible patients included women 18 years of age and older with previously untreated, high-grade serous and/or endometrioid EOC, fallopian tube or primary peritoneal carcinoma who have consented for BRCAm testing. Patients with mucinous, clear cell, low-grade serous or endometrioid, mixed epithelial adenocarcinoma, undifferentiated carcinoma or malignant Brenner’s tumor were excluded. A total of 298 patients were tested for BRCAm for characterization of BRCAm in this population (ClinicalTrials identifier NCT02963688). For a more homogeneous group to assess accurate results for survival analysis and treatment response, survival analysis was performed in the patient group after excluding patients who received neoadjuvant chemotherapy or secondary debulking surgery, patients with stages I–II, and patients who did not receive primary chemotherapy. All patients were provided with written informed consent on the protocol approved by the Institutional Review Board.

2.2 | Clinical characteristics assessment

Epithelial ovarian cancer patients routinely underwent primary cytoreductive surgery followed by six to nine cycles of adjuvant intravenous taxane and platinum-based chemotherapy, or patients were treated with three cycles of neoadjuvant chemotherapy followed by interval debulking surgery and an additional three to six cycles of taxane and platinum-based chemotherapy according to the physician’s clinical decision. After initial treatment, patients were routinely followed up every 3 months for the first year, then...
565 ovarian cancer cases (KGOG3019)

298 cases of high-grade serous / endometrioid ovarian cancer cases

BRCA germline mutation testing (n=298)

BRCA germline mutation Positive (n=78, 26.2%)

BRCA germline mutation Negative (n=220, 73.8%)

BRCA somatic mutation testing (n=86)

BRCA somatic mutation Positive (n=11, 12.8%)

Survival analysis (n=181)

FIGURE 1 Flowchart outlining the study

every 6 months for up to 5 years, and annually thereafter. Patients were monitored based on clinical symptoms, laboratory tests, and imaging examinations. For treatment for recurrent EOC, a taxane and platinum-based regimen was used for the platinum-sensitive group and a nonplatinum-based chemotherapy regimen (topotecan/belotucan, pegylated liposomal doxorubicin, etoposide, and others) was used for the platinum-resistant group. Since PARPi was not used as standard treatment during the period in which the study subjects were being treated, chemotherapy was the main treatment. There was no difference in treatment regimen for recurrence between patients with and without BRCAm in this study.

Clinical and pathologic data were collected from the KGOG 3019 database and patient medical records: family cancer history for second-degree relevant, personal cancer history, age at diagnosis, surgical outcome at primary cytoreductive surgery/interval debulking surgery, tumor histology, stage of disease, chemotherapy regimen (first and subsequent lines of treatment), and response assessments including CA-125 level/imaging results.

Surgical staging was assessed according to the International Federation of Gynecologist and Obstetricians (FIGO) staging system when diagnosed. Surgical outcomes were categorized as residual disease of 0-10 mm as optimal and >10 mm as suboptimal after debulking surgery. Platinum sensitivity was classified as two groups: platinum sensitive and platinum resistant. Regarding sensitivity for platinum chemotherapy, platinum sensitive was defined as more than 6 months of platinum-free interval and platinum resistance was defined as less than 6 months of platinum-free interval after the first line of adjuvant chemotherapy.

Progression-free survival was defined as the interval between histologic diagnosis and first progression, death as a result of disease, or last follow-up. Overall survival (OS) was defined as the interval between histologic diagnosis and death as a result of disease, or last follow-up. Death as a result of nondisease-related causes was not considered in the calculation of PFS or OS. Disease progression was determined based on CA-125 levels and imaging results according to Gynecologic Cancer Inter Group (GCIG) modification of the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines modified for ovarian cancer and RECIST criteria. Overall response rate (ORR) was defined as the proportion of patients who had a partial or complete response to therapy. It does not include stable disease and is a direct measure of drug tumoricidal activity.
2.3 | DNA extraction

DNA was extracted using from the EDTA-anticoagulated whole blood using the Wizard® Genomic DNA Purification Kit according to the manufacturer’s instructions (Promega). Surgical snap-frozen fresh tissue was used for BRCAm detection. Genomic DNA was extracted by the concentration of extracted DNA, and it was measured using a Pico-Green dsDNA assay (Quanti-TTM PicoGreen® dsDNA kit, Invitrogen).

2.4 | Targeted sequencing and variant calling (germline DNA and tumor DNA)

All coding exons and intron sequences of 20 bp around each exon were targeted in the BRCA1 and BRCA2 genes, ultimately resulting in a total size of 22,462 bp. Briefly, 30 ng of DNA (10 ng per pool) was amplified using the Ion AmpliSeq BRCA1 and BRCA2 Panel (Life Technologies) containing 167 primer pairs and an Ion AmpliSeq kit 2.0. The amplicons were ligated to adapters with barcodes of the Ion Xpress Barcode Adapter Kit (Life Technologies). The libraries with adapters were pooled for multiplexing. The amplicons were clonally amplified through emulsion PCR by using a IT OneTouch Template Kit 2.0 on an IT OneTouch system (Life Technologies) following the manufacturer’s instructions. Template-positive Ion Sphere Particles (ISPs) were enriched, and purified ISPs were loaded on an Ion 316 or 318 Chip. Twelve barcoded samples on an Ion 316 Chip and 32 barcoded samples on a 318 Chip were sequenced. Targeted sequencing was performed using the Ion PGM platform using an Ion PGM sequencing 200 kit following the manufacturer’s instructions.

2.5 | Bioinformatic analysis and mutation prioritization

Data analysis including signal processing, base calling, read alignment, and coverage analysis was done using Torrent Suite software (ver 4.0.3, Life Technologies). Torrent browser plugin software such as variantCaller (v4.2.1.0) and coverageAnalysis (v4.2.1.4) was used for variant calling and coverage analysis. The variant calling was performed using the “Germ Line – PGM – High Stringency” setting. The variants were functionally annotated using the ANNOVAR tool.28,29 A number of in silico tools, including SIFT, Polyphen 2, LRT, MutationTaster, MutationAssessor, and FATHMM, GERP score, were used.29 To prioritize the pathogenic mutations, we applied the criteria for pathogenicity classification according to the American College of Medical Genetics and Genomics (ACMG) guideline, and the prioritized variants were classified as pathogenic variants and likely pathogenic variants.30 “Disease-causing mutations” (DM) in the Human Genome Mutation Database (HGMD, professional version 2014.01), “pathogenic” mutations in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), or causal mutation in UMD (http://www.umd.be/BRCA1/, http://www.umd.be/BRCA2/) were categorized as known mutations.31,32 The “A” of the ATG translation initiation codon is described as position number 1 in the BRCA1 (NM_007294.2) and BRCA2 (NM_000059.3).

2.6 | Sanger sequencing

All of the prioritized mutations were validated with independent Sanger sequencing. The mutated DNA was amplified by PCR using primer pairs designed with Primer3 software. Relevant regions were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems Foster City).

2.7 | Statistical analysis

Frequency distributions between categorical variables among the groups were compared using the $\chi^2$ test. The Fisher’s exact test was used if the expected frequency was <5. The Kaplan-Meier method with the log-rank test was used to estimate and compare PFS and OS. The Cox proportional hazard model was used to evaluate the association between factors and survival. Statistical analyses were performed by SPSS software (version 21.0). A $P$ value of ≤.05 was considered statistically significant, and all $P$ values were two-sided.

3 | RESULTS

A flowchart outlining the study is shown in Figure 1. A total of 565 patients were enrolled to the KGOG 3019 from 2010 to 2015, and 298 patients were eligible for the current study: patients with EOC, peritoneal or fallopian tube primary tumor with high-grade serous or endometrioid cell type and consent to examine BRCA genotyping. Median follow-up for all patients was 28.1 months.

3.1 | Patient characteristics

The patient characteristics of the current study and BRCAm status are shown in Table 1. An insignificant association between BRCAm(+) and BRCAm(-) patients for age, primary site, and FIGO stage was observed in our study. The median age at diagnosis for BRCAm(+) EOC patients was 53.0 years (range 38–75 years) and was similar to BRCAm(-) patients (median 53.0 years, range 30–79 years). This was different from previous studies, which showed earlier onset of disease with BRCAm, in particularly patients with gBRCA1m.10,15,33-35 Most BRCAm(+) were found in high-grade serous EOC (98.9%). Sixteen patients had a personal history of breast cancer and this was significantly higher in the BRCAm(+) group (10/89, 11.2%) than in the BRCAm(-) group (6/209, 2.9%, $P = .008$; Table 1). This means 62.5% (10/16) showed BRCAm(+) among patients with EOC having a personal history of breast
cancer. Number of breast cancer family history was significant different between BRCAm(+) and BRCAm(-) patients (19/89, 21.3% vs 17/209, 8.1%, \(P = .003\); Table 1), showing a 52.8% (19/36) probability of gBRCAm in EOC patients with family breast cancer history. Number of ovarian cancer family history was very strongly associated with BRCAm(+), 15.7% (14/89) of BRCAm(+) and 3.3% (7/209) of BRCAm(-), respectively (\(P < .001\)), also showing a 66.7% (14/21) chance of BRCAm.

In the present study, regarding optimality, described as residual disease after debulking surgery, a residual tumor less than 1 cm after primary debulking surgery was observed in 80.2% of patients. There were no significant differences between BRCAm status and optimal primary debulking rate (BRCAm(+) vs BRCAm(-), 77.5% vs 81.3%, \(P = .070\); Table 1) and sensitivity to first-line platinum-based combination chemotherapy (BRCAm(+) vs BRCAm(-), 63.6% vs 50.0, \(P = .106\); Table 1), which is not consistent with previous studies.\(^{10,36}\)

**TABLE 1** Patient characteristics

| Characteristics                        | Total     | BRCAm(-)  | BRCAm(+)  | gBRCA1m(+) | gBRCA2m(+) | sBRCAm(+) | \(P\) value* |
|----------------------------------------|-----------|-----------|-----------|------------|------------|-----------|-------------|
| Total cases, n (%)                     | 298       | 209       | 89        | 64         | 14         | 11        | .712        |
| Age at diagnosis, years, median (range)| 53 (30-79)| 53 (30-79)| 53 (38 – 75)| 53 (38-73)| 57 (44-75)| 54 (48-71)|            |
| Primary site, n (%)                    |           |           |           |            |            |           |            |
| Ovary                                  | 270 (90.6)| 188 (90.0)| 82 (92.1)| 58 (90.6)| 13 (92.9)| 11 (100)| .438        |
| Peritoneum                             | 14 (4.7)  | 12 (5.7)  | 2 (2.2)   | 2 (3.1)   | 0          | 0         |            |
| Fallopian tube                         | 14 (4.7)  | 12 (5.7)  | 5 (5.6)   | 4 (6.3)   | 1 (7.1)   | 0         |            |
| Histology, n (%)                       |           |           |           |            |            |           |            |
| Serous                                 | 282 (94.7)| 194 (92.8)| 88 (98.9)| 63 (98.4)| 14 (100)| 11 (100)| .046        |
| Endometrioid                           | 16 (5.4)  | 15 (7.2)  | 1 (1.1)   | 1 (1.6)   | 0          | 0         |            |
| FIGO stage, n (%)                      |           |           |           |            |            |           |            |
| I-II                                   | 34 (11.4) | 28 (13.4) | 6 (6.7)   | 4 (6.3)   | 1 (7.1)   | 1 (9.1)  | .113        |
| III-IV                                 | 264 (94.3)| 181 (86.6)| 83 (93.3)| 60 (93.7)| 13 (92.9)| 10 (90.9)|            |
| Personal breast cancer history, n (%)  |           |           |           |            |            |           |            |
| No                                     | 282 (94.6)| 203 (97.1)| 79 (88.8)| 56 (87.5)| 12 (85.7)| 11 (100)| .008        |
| Yes                                    | 16 (5.4)  | 6 (2.9)   | 10 (11.2)| 8 (12.5)| 2 (14.3)| 0         |            |
| Family breast cancer history, n (%)    |           |           |           |            |            |           |            |
| Unknown                                | 28 (9.4)  | 23 (11.0)| 5 (5.6)   | 2 (3.1)   | 3 (21.4)| 0         | .003        |
| No                                     | 234 (78.5)| 169 (80.9)| 65 (73.0)| 46 (71.9)| 9 (64.3)| 10 (90.9)|            |
| Yes                                    | 36 (12.1)| 17 (8.1)  | 19 (21.3)| 16 (25.0)| 2 (14.3)| 1 (9.1)  |            |
| Family ovarian cancer history, n (%)   |           |           |           |            |            |           |            |
| Unknown                                | 28 (9.4)  | 23 (11.0)| 5 (5.6)   | 2 (3.1)   | 3 (21.4)| 0         | <.001       |
| No                                     | 249 (83.6)| 179 (85.6)| 70 (78.7)| 49 (76.6)| 10 (71.4)| 11 (100)|            |
| Yes                                    | 21 (7.0)  | 7 (3.4)   | 14 (15.7)| 13 (20.3)| 1 (7.1)| 0         |            |
| Residual disease after debulking surgery|          |           |           |            |            |           |            |
| 0-10 mm                                | 239 (80.2)| 170 (81.3)| 69 (77.5)| 54 (84.4)| 9 (64.3)| 6 (54.5)| .070        |
| >10 mm                                 | 45 (15.1) | 33 (15.8)| 12 (13.5)| 5 (7.8)   | 2 (14.3)| 5 (45.5)|            |
| Missing data                           | 14 (4.7)  | 6 (2.9)   | 8 (9.0)   | 5 (7.8)   | 3 (21.4)| 0         |            |
| Platinum sensitivity (N = 181), n (%)  |          |           |           |            | n = 126  | n = 55 | .106        |
| Sensitive                              | 98 (54.1)| 63 (50.0)| 35 (63.6)| 24 (37.5)| 8 (57.1)| 3 (27.3)|            |
| Resistant                              | 83 (45.9)| 63 (50.0)| 20 (36.4)| 14 (21.8)| 2 (14.2)| 4 (36.4)|            |

Note: Table shows column percentages.
Abbreviations: BRCAm, BRCA mutation; FIGO, International Federation of Gynecology and Obstetrics.

*\(P\) value of Fisher’s exact test and chi-square test between the BRCAm(+) and BRCAm(-) groups. **Platinum sensitivity was assessed from available data of 181 patients of advanced ovarian cancer patients who had undergone adjuvant platinum-based chemotherapy.
3.2 | Prevalence of BRCA1/2 mutations

In the present study, germline mutation was observed in 26.2% of patients and somatic mutation in gBRCAm(-) specimens was observed in 12.8% (Table 1). All 298 patients were tested for gBRCAm. A total of 49 different mutations were identified in 78 patients. The germline mutation profile of 78 patients is shown in Table 2. The ratio of gBRCA1m:gBRCA2m was 4.61 (64 vs 14 patients). Regarding patterns of mutation types in the study population, stopgain was the most common, with 31 patients (39.7%), followed by frameshift insertion (16 patients, 20.5%), frameshift deletion (16 patients, 20.5%), splicing (nine patients, 11.5%), and missense (six patients, 7.7%). Seven of these mutations were novel: p.P1238fs, p.N941fs, p.G394fs, p.G1319fs in BRCA1 and p.D2110fs, p.S1992fs, p.K3084fs in BRCA2. Twelve recurrent mutations were identified: 11 in BRCA1 and one in BRCA2. Five of them (p.Y130X [n = 3], p.E1210fs [n = 9], p.Q1144X [n = 3], p.W1815X [n = 3], and c.5467+1G>A [n = 5] in BRCA1) were found in at least three unrelated patients. Haplotype analysis demonstrated no founder effects in three recurrent mutations such as p.Y130X, p.E1210fs, and c.5467+1G>A in BRCA1. All of the haplotypes were exclusively seen only in mutation-containing cases. Frequently occurring BRCA mutation loci are shown in Table S1.

The number of mapped reads and percent base reads on target showed medians of 125,340 and 95%, respectively. The average depth of coverage was 675-fold per sample (range of 264- to 1983-fold). A total of 100% and 99.4% of bases were covered by at least 1-fold and 20-fold of coverage, respectively. A total of 153 variants were found in 298 cases. Among them, a total of 53 variants were prioritized, according to the ACMG criteria. In total, 49 mutant alleles including 36 pathogenic and 13 likely pathogenic mutations were validated by Sanger sequencing (validation rate of 92%).

The sBRCAm were tested in 86 patients without gBRCAm and available fresh tumor samples (Figure 1). Eleven (12.8%) patients had sBRCAm only among 86 gBRCAm negative specimens and the somatic BRCA mutation rate was estimated to be 3.7% among all patients. The gene profile of sBRCAm patients is shown in Table 2. All sBRCAm were found in serous-type only and the ratio of sBRCA1m (n = 8) to sBRCA2m (n = 3) was 2.7:1.

3.3 | Survival outcomes

For more a homogenous group to assess the accurate results for survival analysis and treatment response, we excluded patients who had undergone neoadjuvant chemotherapy or secondary debulking surgery, patients with stages I-II, and patients who did not receive primary chemotherapy (Figure 1). We finally analyzed a subset of 181 cases for survival analysis. With a median follow-up duration of 28.1 months (range 1.9-89.7) from initial diagnosis, 45 patients died of EOC (seven patients in the BRCAm(+) group and 38 patients in the BRCAm(-) group). The estimated 5-year OS was 64.6% using the Kaplan-Meier method.

For survival outcomes, Kaplan-Meier curves for PFS and OS are shown in Figure 2. The BRCAm(+) group showed better PFS and OS, but only OS with significance (P = .004). In univariate analysis for OS for this cohort, BRCAm(-) was associated with significantly worse OS compared to cases with BRCAm (P = .021, hazard ratio = 10.620, 95% confidence interval [CI] = 1.436-78.555). The other characteristics significantly related to OS were residual disease after debulking surgery (P = .050) and platinum sensitivity (P < .001). The results for median and 5-year survival for PFS and OS by clinical variables using the log-rank test are shown in Table S2. In multivariate analysis for OS, the presence of BRCAm was significantly associated with OS (P = .049, hazard ratio = 7.637, CI = 1.010-58.278; Table 3) in addition to platinum sensitivity (P < .001, hazard ratio = 11.254, CI = 4.333 - 29.232) indicating it is an independent prognostic factor for survival regardless of platinum sensitivity to first-line chemotherapy. The location of BRCAm was not associated with survival outcome (Figure S1).

3.4 | Treatment response

The number of patients by the chemotherapy regimen type (platinum-based/non platinum-based) in BRCAm(+) and BRCAm(-) group is shown in Table S3. There was no difference in treatment regimen between patients with BRCAm(+) and BRCAm(-) for the second (P = .272) and third (P = .316) regimens in this study. The ORR of first-line to third-line chemotherapy of patients was assessed by BRCAm status (Figure 3 and Table S3). There was no statistical difference in ORR in first-line chemotherapy between BRCAm(+) and BRCAm(-) patients (P = .838; Figure 3). Among these patients, 97 had first recurrent disease and 86 of 97 patients received second-line chemotherapy. Second-line chemotherapy revealed a higher response in BRCAm(+) patients compared to BRCAm(-) patients (60.0% vs 34.5%, P = .065; Figure 3). Among these patients, 52 patients had second recurrent disease and 39 of these 52 patients received third-line chemotherapy. Importantly, third-line chemotherapy showed significantly improved ORR in BRCAm(+) patients compared to BRCAm(-) patients (66.7% vs 13.3%, P = .004; Figure 3). BRCAm carriers have been shown to have a better response to platinum-based chemotherapy, and our results showed a trend of patients with BRCAm maintaining a higher response rate to platinum treatment along the progressed chemotherapy lines compared to patients without BRCAm. Thus, the survival benefit of patients with BRCAm for OS might be associated with maintained platinum sensitivity after first-line platinum-based treatment. Although the sensitivity to first-line platinum-based chemotherapy was similar in both BRCAm(+) and BRCAm(-) groups, successive lines showed a trend of better treatment response for BRCAm(+) patients in our results.
# Germline mutation profile of 78 patients and somatic mutation profile of 11 patients

| Case | Exon/intron | Gene   | RefSeq | Exon | NT alteration | AA alteration | Type   | Classification |
|------|-------------|--------|--------|------|---------------|---------------|--------|----------------|
| 8    | Exonic      | BRCA2  | NM_000059 | Exon15 | c.7480C>T    | p.R2494X      | Stopgain | Pathogenic     |
| 12   | Exonic      | BRCA2  | NM_000059 | Exon11 | c.6331dupA   | p.R2112fs     | Frameshift deletion | Likely pathogenic |
| 14   | Exonic      | BRCA1  | NM_007294 | Exon6  | c.390C>A     | p.Y130X       | Stopgain | Pathogenic     |
| 15   | Exonic      | BRCA1  | NM_007294 | Exon10 | c.2433delC   | p.K812fs      | Stopgain | Pathogenic     |
| 22   | Exonic      | BRCA1  | NM_007294 | Exon22 | c.5445G>A    | p.W1815X      | Stopgain | Pathogenic     |
| 24   | Exonic      | BRCA1  | NM_007294 | Exon10 | c.3289delA   | p.S1097fs     | Frameshift deletion | Pathogenic |
| 25   | Exonic      | BRCA1  | NM_007294 | Exon10 | c.2354T>A    | p.L785X       | Stopgain | Pathogenic     |
| 32   | Exonic      | BRCA1  | NM_007294 | Exon10 | c.2433delC   | p.K812fs      | Stopgain | Pathogenic     |
| 33   | Splicing    | BRCA1  | NM_007294 | IVS23  | NA           | Splicing      | Pathogenic |
| 34   | Exonic      | BRCA1  | NM_007294 | Exon19 | c.5266C>T    | p.Q1756X      | Stopgain | Pathogenic     |
| 37   | Exonic      | BRCA2  | NM_000059 | Exon10 | c.1399A>T    | p.K467X       | Stopgain | Pathogenic     |
| 38   | Exonic      | BRCA1  | NM_007294 | Exon10 | c.3710_3711dup | p.P1238fs    | Frameshift deletion | Likely pathogenic |
| 44   | Splicing    | BRCA1  | NM_007294 | IVS19  | NA           | Splicing      | Pathogenic |
| 52   | Exonic      | BRCA1  | NM_007294 | Exon6  | c.390C>A     | p.Y130X       | Stopgain | Pathogenic     |
| 55   | Exonic      | BRCA1  | NM_007294 | Exon16 | c.5030_5033del | p.T1677fs    | Frameshift deletion | Pathogenic |
| 56   | Exonic      | BRCA1  | NM_007294 | Exon10 | c.3627dupA   | p.E1210fs     | Frameshift deletion | Pathogenic |
| 62   | Exonic      | BRCA1  | NM_007294 | Exon10 | c.3627dupA   | p.E1210fs     | Frameshift deletion | Pathogenic |
| 64   | Exonic      | BRCA1  | NM_007294 | Exon21 | c.5339T>C    | p.L1780P      | Missense | Likely pathogenic |
| 65   | Splicing    | BRCA1  | NM_007294 | IVS23  | NA           | Splicing      | Pathogenic |
| 69   | Exonic      | BRCA2  | NM_000059 | Exon11 | c.5975_5976dupCA | p.L1993fs  | Frameshift deletion | Likely pathogenic |
| 71   | Exonic      | BRCA1  | NM_007294 | Exon9  | c.616C>T     | p.Q206X       | Stopgain | Pathogenic     |
| 78   | Exonic      | BRCA1  | NM_007294 | Exon10 | c.1936delA   | p.S646fs      | Frameshift deletion | Pathogenic |
| 83   | Exonic      | BRCA1  | NM_007294 | Exon10 | c.3627dupA   | p.E1210fs     | Frameshift deletion | Pathogenic |
| 90   | Exonic      | BRCA1  | NM_007294 | Exon10 | c.3991C>T    | p.Q1331X      | Stopgain | Pathogenic     |
| 91   | Exonic      | BRCA1  | NM_007294 | Exon21 | c.5339T>C    | p.L1780P      | Missense | Likely pathogenic |
| 93   | Exonic      | BRCA1  | NM_007294 | Exon21 | c.5339T>C    | p.L1780P      | Missense | Likely pathogenic |
| 94   | Exonic      | BRCA1  | NM_007294 | Exon22 | c.5445G>A    | p.W1815X      | Stopgain | Pathogenic     |
| 97   | Exonic      | BRCA1  | NM_007294 | Exon16 | c.5030_5033del | p.T1677fs    | Frameshift deletion | Pathogenic |
| 99   | Splicing    | BRCA1  | NM_007294 | IVS23  | NA           | Splicing      | Pathogenic |
| 107  | Exonic      | BRCA1  | NM_007294 | Exon17 | c.5080G>T    | p.E1694X      | Stopgain | Pathogenic     |
| 115  | Exonic      | BRCA1  | NM_007294 | Exon6  | c.390C>A     | p.Y130X       | Stopgain | Pathogenic     |
| 119  | Exonic      | BRCA1  | NM_007294 | Exon10 | c.3627dupA   | p.E1210fs     | Frameshift deletion | Pathogenic |
| 128  | Exonic      | BRCA2  | NM_000059 | Exon11 | c.6724_6725del | p.D2242fs    | Frameshift deletion | Pathogenic |

(Continues)
| Case | Exon/intron | Gene | RefSeq | Exon | NT alteration | AA alteration | Type | Classification |
|------|-------------|------|--------|------|--------------|--------------|------|----------------|
| 134  | Exonic      | BRCA1| NM_007294 | Exon10 | c.1480C>T | p.Q494X | Stopgain | Pathogenic |
| 136  | Splicing    | BRCA1| NM_007294 | IVS6  | c.302-2A>C | NA | Splicing | Pathogenic |
| 137  | Splicing    | BRCA1| NM_007294 | IVS23 | c.5467+1G>A | NA | Splicing | Pathogenic |
| 140  | Exonic      | BRCA1| NM_007294 | Exon10 | c.3627dupA | p.E1210fs | Frameshift insertion | Pathogenic |
| 149  | Exonic      | BRCA1| NM_007294 | Exon10 | c.1831delC | p.L611X | Stopgain | Pathogenic |
| 154  | Exonic      | BRCA1| NM_007294 | Exon6  | c.390C>A | p.Y130X | Stopgain | Pathogenic |
| 155  | Splicing    | BRCA1| NM_007294 | IVS23 | c.5467+1G>A | NA | Splicing | Pathogenic |
| 156  | Exonic      | BRCA2| NM_000059 | Exon16 | c.7673_7674del | p.E2558fs | Frameshift deletion | Pathogenic |
| 159  | Exonic      | BRCA1| NM_007294 | Exon10 | c.2740G>T | p.E914X | Stopgain | Pathogenic |
| 164  | Exonic      | BRCA1| NM_007294 | Exon10 | c.3627dupA | p.E1210fs | Frameshift insertion | Pathogenic |
| 165  | Exonic      | BRCA1| NM_007294 | Exon10 | c.3430C>T | p.Q1144X | Stopgain | Pathogenic |
| 170  | Exonic      | BRCA1| NM_007294 | Exon10 | c.2354T>A | p.L785X | Stopgain | Pathogenic |
| 177  | Exonic      | BRCA1| NM_007294 | Exon10 | c.928C>T | p.Q310X | Stopgain | Pathogenic |
| 180  | Exonic      | BRCA2| NM_000059 | Exon11 | c.2830A>T | p.K944X | Stopgain | Pathogenic |
| 186  | Exonic      | BRCA1| NM_007294 | Exon22 | c.5445G>A | p.W1815X | Stopgain | Pathogenic |
| 187  | Splicing    | BRCA1| NM_007294 | IVS17 | c.5074+1G>T | NA | Splicing | Pathogenic |
| 188  | Exonic      | BRCA1| NM_007294 | Exon10 | c.2821_2822dup | p.N941fs | Frameshift insertion | Likely pathogenic |
| 189  | Exonic      | BRCA1| NM_007294 | Exon10 | c.1179_1180dupAG | p.G394fs | Frameshift insertion | Likely pathogenic |
| 201  | Exonic      | BRCA1| NM_007294 | Exon10 | c.3627dupA | p.E1210fs | Frameshift insertion | Pathogenic |
| 202  | Exonic      | BRCA1| NM_007294 | Exon17 | c.5080G>T | p.E1694X | Stopgain | Pathogenic |
| 213  | Exonic      | BRCA1| NM_007294 | Exon21 | c.5339T>C | p.L1780P | Missense | Likely pathogenic |
| 217  | Exonic      | BRCA1| NM_007294 | Exon10 | c.3442delG | p.E1148fs | Frameshift deletion | Pathogenic |
| 223  | Exonic      | BRCA1| NM_007294 | Exon21 | c.5339T>C | p.L1780P | Missense | Likely pathogenic |
| 225  | Exonic      | BRCA1| NM_007294 | Exon10 | c.3296delC | p.P1099fs | Frameshift deletion | Pathogenic |
| 228  | Exonic      | BRCA1| NM_007294 | Exon10 | c.3718C>T | p.Q1240X | Stopgain | Pathogenic |
| 229  | Exonic      | BRCA1| NM_007294 | Exon10 | c.3627dupA | p.E1210fs | Frameshift insertion | Pathogenic |
| 233  | Exonic      | BRCA1| NM_007294 | Exon10 | c.2359delG | p.E787fs | Frameshift deletion | Pathogenic |
| 244  | Exonic      | BRCA2| NM_000059 | Exon11 | c.4471_4474del | p.L1491fs | Frameshift deletion | Pathogenic |
| 245  | Exonic      | BRCA1| NM_007294 | Exon11 | c.4117G>T | p.E1373X | Stopgain | Pathogenic |
| 249  | Exonic      | BRCA2| NM_000059 | Exon11 | c.2798_2799del | p.T933fs | Frameshift deletion | Pathogenic |
| 250  | Splicing    | BRCA2| NM_000059 | Exon24_ | c.9254_9256+11del | NA | Splicing | Likely pathogenic |
| 254  | Exonic      | BRCA1| NM_007294 | Exon10 | c.3627dupA | p.E1210fs | Frameshift insertion | Pathogenic |
| 255  | Exonic      | BRCA1| NM_007294 | Exon10 | c.3954dupT | p.G1319fs | Frameshift insertion | Likely pathogenic |
DISCUSSION

This study was a large-scale nationwide next-generation sequencing (NGS)-based germline and somatic BRCA mutation prevalence study with clinical outcomes including treatment response in Korean ovarian cancer patients regardless of family history and age at diagnosis. We conducted germline and somatic BRCA testing with whole blood and fresh frozen samples, respectively, using NGS analysis. The combined prevalence of BRCA germline and somatic mutation in patients with EOC differed depending on the sample cohort and detection method. The prevalence of germline or somatic BRCA mutations in epithelial ovarian cancer with all histologic types is known to vary from 5% to 29% by ethnicity and country. The reported frequency of BRCAm in patients with high-grade serous EOC varies between 19% and 30%. Our results showed higher rate of combining gBRCA (26.2%) in high-grade serous EOC patients and sBRCA (12.8%) among patients without germline mutation in Korean patients with high-grade serous EOC compared to previous reports. Although our study had potential selection biases similar to the TCGA ovarian cohort, which may have resulted in various

| Table 2 (Continued) |

| Germline mutation profile (n = 78) |
|---|---|---|---|---|---|---|
| Case | Exon/intron | Gene | RefSeq | Exon | NT alteration | AA alteration | Type | Classification |
| 256 | Exonic | BRCA1 | NM_007294 | Exon10 | c.3430C>T | p.Q1144X | Stopgain | Pathogenic |
| 274 | Exonic | BRCA1 | NM_007294 | Exon10 | c.3430C>T | p.Q1144X | Stopgain | Pathogenic |
| 276 | Exonic | BRCA2 | NM_000059 | Exon14 | c.7375A>T | p.K2459X | Stopgain | Pathogenic |
| 278 | Exonic | BRCA2 | NM_000059 | Exon11 | c.6600_6601del | p.S2201X | Stopgain | Pathogenic |
| 280 | Exonic | BRCA1 | NM_007294 | Exon23 | c.5470_5477del | p.I1824fs | Frameshift deletion | Pathogenic |
| 290 | Exonic | BRCA2 | NM_000059 | Exon10 | c.1796_1800del | p.S599X | Stopgain | Pathogenic |
| 294 | Exonic | BRCA1 | NM_007294 | Exon6 | c.390C>A | p.Y130X | Stopgain | Pathogenic |
| 295 | Exonic | BRCA1 | NM_007294 | Exon21 | c.5339T>C | p.L1780P | Missense | Likely pathogenic |
| 297 | Exonic | BRCA1 | NM_007294 | Exon10 | c.3895C>T | p.Q1299X | Stopgain | Pathogenic |
| 298 | Exonic | BRCA1 | NM_007294 | Exon10 | c.3954dupT | p.G1319fs | Frameshift insertion | Likely pathogenic |
| 299 | Exonic | BRCA2 | NM_000059 | Exon11 | c.2798_2799del | p.T933fs | Frameshift deletion | Pathogenic |
| 302 | Exonic | BRCA1 | NM_007294 | Exon10 | c.3442delG | Frameshift deletion | Pathogenic |

| Somatic mutation profile (n = 11) |
|---|---|---|---|---|---|---|
| Case | Exon/intron | Gene | RefSeq | Exon | NT alteration | AA alteration | Type | Classification |
| 3 | Exonic | BRCA1 | NM_007294 | Exon10 | c.3449delC | p.P1150fs | Frameshift deletion | Likely pathogenic |
| 5 | Splicing | BRCA1 | NM_007294 | IVS22 | c.5404_5406+1del | NA | Splicing | Likely pathogenic |
| 7 | Exonic | BRCA2 | NM_000059.3 | Exon11 | c.5073delA | p.K1691fs | Frameshift deletion | Pathogenic |
| 19 | Exonic | BRCA2 | NM_000059.3 | Exon23 | c.8991T>G | p.Y2997X | Stopgain | Pathogenic |
| 40 | Exonic | BRCA1 | NM_007294 | Exon21 | c.5339T>C | p.L1780P | Missense | Likely pathogenic |
| 43 | Exonic | BRCA2 | NM_000059.3 | Exon11 | c.5576_5579delTTAA | p.I1859fs | Frameshift deletion | Pathogenic |
| 45 | Splicing | BRCA1 | NM_007294 | IVS23 | c.5467+1G>A | NA | Splicing | Pathogenic |
| 47 | Exonic | BRCA1 | NM_007294 | Exon10 | c.2467A>T | p.R823X | Stopgain | Likely pathogenic |
| 67 | Exonic | BRCA1 | NM_007294 | Exon4 | c.160C>T | p.Q54X | Stopgain | Pathogenic |
| 74 | Exonic | BRCA1 | NM_007294 | Exon10 | c.3252delT | p.R1085fs | Frameshift deletion | Likely pathogenic |

Note: The “A” of the ATG translation initiation codon is described as position number 1 in the BRCA1 (NM_007294.2) and BRCA2 (NM_000059.3). According to the American College of Medical Genetics and Genomics (ACMG) guideline, the prioritized variants were classified as pathogenic variants and likely pathogenic variants. “Disease-causing mutations” (DM) in THE Human Genome Mutation Database (HGMD, professional version 2014.01), “pathogenic” mutations in ClinVar, or causal mutation in UMD were categorized as known mutations.
Abbreviations: AA, amino acid; NA, not applicable; NT, nucleotide.

4 | DISCUSSION

This study was a large-scale nationwide next-generation sequencing (NGS)-based germline and somatic BRCA mutation prevalence study with clinical outcomes including treatment response in Korean ovarian cancer patients regardless of family history and age at diagnosis. We conducted germline and somatic BRCA testing with whole blood and fresh frozen samples, respectively, using NGS analysis. The combined prevalence of BRCA germline and somatic mutation in patients with EOC differed depending on the sample cohort and detection method. The prevalence of germline or somatic BRCA mutations in epithelial ovarian cancer with all histologic types is known to vary from 5% to 29% by ethnicity and country. The reported frequency of BRCAm in patients with high-grade serous EOC varies between 19% and 30%. Our results showed higher rate of combining gBRCA (26.2%) in high-grade serous EOC patients and sBRCA (12.8%) among patients without germline mutation in Korean patients with high-grade serous EOC compared to previous reports. Although our study had potential selection biases similar to the TCGA ovarian cohort, which may have resulted in various
estimated for both germline and somatic mutation rates,\textsuperscript{40} it gives important message that we should perform tumor BRCA testing to provide a chance to use PARPi, even in this BRCAm (-) population.

The proportion of gBRCA1m to gBRCA2m in our cohort was reported as 4.6:1. Previous studies in predominantly Caucasian population-based cohorts showed proportions of 1.7:1 in Australia,\textsuperscript{10} 1.9:1 in Canada,\textsuperscript{41} and 1.7:1 to in the United States.\textsuperscript{42} In Asian populations, most studies have reported that BRCA1 comprised a higher proportion of BRCA mutations than BRCA2.\textsuperscript{18,19,21,22,43,44} The high ratio for BRCA1 to BRCA2 mutation (4.6:1) in this study was interesting, but a larger study may be needed in the future. Founder mutations were shown in various ethnic groups, including Ashkenazi Jews, Icelanders, Russians, and Israelis.\textsuperscript{45-47} Until recently, no founder mutations had been reported in Asian populations.\textsuperscript{17-20,22,43} In this study, we also did not identify founder mutations. Therefore, genetic testing of BRCA1m and BRCA2m using full sequencing may

**TABLE 3** Univariate and multivariate Cox proportional hazards analysis for overall survival

| Variables                                      | OS                  | Univariate HR (95% CI) | P value | Multivariate HR (95% CI) | P value |
|------------------------------------------------|---------------------|------------------------|---------|--------------------------|---------|
| Age at diagnosis, years, continuous            |                     | 1.034 (0.995 - 1.076)  | .092    |                          |         |
| BRCA mutation ([+] vs [-])                     |                     | 10.620 (1.436 - 78.555)| .021    | 7.637 (1.010 - 58.278)   | .049    |
| Residual disease after debulking surgery (0-10 mm vs >10 mm) |                     | 2.330 (1.001 - 5.422)  | .050    | 2.396 (0.938 - 6.118)    | .068    |
| Platinum response (sensitive vs resistant)     |                     | 9.914 (4.094 - 24.006 | <.001   | 11.254 (4.333 - 29.232)  | <.001   |

Abbreviations: CI, confidential interval; HR, hazard ratio; OS, overall survival.
be beneficial in Korean patients until highly recurrent founder mutations specific to Koreans have been identified. Wu et al.\(^\text{18}\) reported that 5% of their observed $gBRCA_m$ was large genomic rearrangement. Therefore, there is the possibility of undetected genomic rearrangement mutations in our study. However, we confirmed that large genomic rearrangement of the $BRCA$ gene was very rare in Korean $BRCA$ mutation carriers (<1%) in a previous report and it may not change the mutation rate in this study.

Korean data for $BRCA$ mutation in EOC patients have been published and showed the clinical and genetic characteristics of this group.\(^\text{48}\) There are substantial differences between the previous and current study that current study provides more information regarding the treatment response of patients’ group. While the previous study showed only the characteristics of EOC patients with $BRCA_m$, our study also showed the results of survival data and response to first-, second-, and third-line chemotherapies. In the previous study, the frequency of $BRCA_m$ was 16.5%, which is different to the 26.2% found in our study. It can be inferred that the difference in these results may be related to differences in the histologic type. In our study, only serous-type and endometrioid-type EOC were included, and the serous type was 94.7%, but in the previous study, serous type was 72.4%. In addition, the small number of patients may have caused the differences in the results, and a larger data analysis is needed in the future. In both studies, the age at diagnosis was not significant and the family history was a significant factor between the $BRCA_m(+)$ and $BRCA_m(-)$ groups.

Epithelial ovarian cancer patients with $BRCA_m$ were younger than those without $BRCA_m$ in previous reports from Western countries.\(^\text{10}\) However, our data revealed there was no difference in age at diagnosis between the $BRCA_m(+) \text{ and } BRCA_m(-)$ groups (Table 1). In another Asian study, there were similar results for age characteristics in that Asian ovarian cancer patients without $BRCA$ mutation were younger than those in Western countries, and there was no difference in age at diagnosis of ovarian cancer between the $gBRCA_m(+) \text{ and } gBRCA_m(-)$ groups.\(^\text{17,21,43,18}\) In our study, 46 (46/78, 59.0%) patients with $gBRCA_m(+) \text{ had no family history of breast and/or ovarian cancer. These findings indicate that all ovarian patients should be tested for } BRCA_m \text{ for a better treatment decision regardless of their family history.}^{18}$ For the same reason $gBRCA$ testing is currently recommended by the Society of Gynecologic Oncology and the National Comprehensive Cancer Network for all women diagnosed with EOC regardless of family history. Korean national health insurance also supports $BRCA_m$ testing in EOC regardless of family history. Although the relationship between $BRCA$ mutation and advanced stage at diagnosis is controversial, some investigators have reported that ovarian cancers associated with $BRCA_m$ are more likely to be diagnosed at advanced stages although other reports\(^\text{10,20}\) have shown no difference or a higher positive rate at stage I-II.\(^\text{12,42,49,50}\) Our results showed no significant difference in $gBRCA_m$ rate between patients at stage I-II and stage III-IV.

In our study, the presence of $BRCA_m$ was associated with significantly better OS for patient with EOC compared to patients without $BRCA_m$ in univariate and multivariate analysis (Table 3 and Figure 2B), similar to previous reports.\(^\text{10,11,15,36,51}\) $BRCA_m$ is known to be associated with better platinum response.\(^\text{10,36,52}\) Several studies reported higher platinum response in the $gBRCA_m$ patient group even for first-line chemotherapy\(^\text{10,38}\) and this effect may persist in subsequent lines of chemotherapy. Interestingly, our results were slightly different. In first-line chemotherapy, there was no difference platinum response between $BRCA_m(+)$ and $BRCA_m(-)$ patients (Figure 3). In second-line treatment, the platinum response of $BRCA_m(+) \text{ was significantly higher than that of } BRCA_m(-)$. Third-line chemotherapy showed also a significantly higher response in $BRCA_m(+) \text{ patients compared to } BRCA_m(-) \text{ patients. Thus, a trend of maintaining platinum sensitivity along successive lines of platinum chemotherapy in } BRCA_m(+) \text{ patients compared to acquiring resistance to platinum chemotherapy in } BRCA_m(-) \text{ patients was observed. These results are consistent with previous reports apart from no difference of platinum sensitivity at first-line chemotherapy in the current study.}

There were several limitations in our study, including a number of limitations regarding gene analysis. First, we did not analyze a large genomic rearrangement (LGA).\(^\text{12}\) Hasnald et al.\(^\text{43}\) reported that LGA was discovered in patients with negative for $BRCA_m$ using the sequencing mutation test, and the proportion of LGA was approximately 11% of the total $BRCA_m$. An increasing number of LGA are being identified.\(^\text{53}\) Second, analysis was not done.
on whether BRCA gene inactivation was by epigenetic silencing. Consequently, there is a possibility that a proportion of patients with BRCA(m-) might have HR-related gene alterations and they might show similar clinical outcomes, such as platinum sensitivity, to BRCA(m+) patients. Fourth, somatic BRCA mutation was tested only in patients without gBRCAm and with presence of available tissue. The family histories of the patients in this study were retrieved not from a genetic counselling interview but from questionnaire and medical records. In addition, underestimation of the presence of actual familial history cannot be excluded due to separation or loss of families. Therefore, the real proportion of family history in Korean EOC patients may be higher than results in the current study. Finally, the duration of follow-up of patients (28.1 months) was too short to provide accurate survival outcome, and not all enrolled patients were used in survival analysis.

The theory has been persistently raised that the penetrance and biology of BRCAm in Asian patients with EOC differ from that in the Caucasian population, resulting in different presentation of EOC. Furthermore, there is a suggestion that this depends on ethnicity and country. Currently, there are not sufficient data on the risk of EOC among Asian BRCAm carriers, but the risk of breast cancer seems to be lower than in Caucasians. In this study, we demonstrated several differences to Caucasian group, such as comparable or higher prevalence of germline and/or somatic mutations, younger age at diagnosis of ovarian cancer resulting in no difference between age at diagnosis of EOC with or without BRCAm, high ratio of BRCA1m to BRCA2m, and a pattern of platinum sensitivity in the BRCAm group. However, the survival benefit and improved overall platinum sensitivity in the BRCAm group were similar to previous reports involving other ethnicities. Risk for cancer is affected by a number of factors, such as population incidence of cancer, birth cohort, genetic background, and reproductive and lifestyle factors, and these may alter the risk and presentation of EOC in Asian women. There is therefore a need to determine the risk of cancer and calibrate risk assessment methods for Asian women. The current study provides valuable insight and information about the role and presentation of BRCAm in Asian as well as Korean patients with EOC. In a future international clinical trial for high-grade serous EOC, ethnicity including BRCAm and its clinical significance should be considered since this may affect the clinical outcome of investigational treatments.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Huang L, Cronin KA, Johnson KA, Mariotto AB, Feuer EJ. Improved survival time: what can survival cure models tell us about population-based survival improvements in late-stage colorectal, ovarian, and testicular cancer? Cancer. 2008;112:2289-2300.
3. Hanker LC, Loibl S, Burchardt N, et al. The impact of second to sixth line therapy on survival of relapsed ovarian cancer after primary taxane/platinum-based therapy. Ann Oncol. 2012;23:2605-2612.
4. Moore K, Colombo N, Scambia G, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2018;379:2495-2505.
5. Gonzalez-Martín A, Pothuri B, Vergote I, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2019;381:2391-2402.
6. Coleman RL, Fleming GF, Brady MF, et al. Veliparib with first-line chemotherapy and as maintenance therapy in ovarian cancer. N Engl J Med. 2019;381:2403-2415.
7. Pujade-Lauraine E, Ledermann JA, Selle F, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2017;18:1274-1284.
8. Coleman RL, Oza AM, Lorusso D, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390:1949-1961.
9. Mirza MR, Monk BJ, Herrstedt J, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med. 2016;375:2154-2164.
10. Alsop K, Fereday S, Meldrum C, et al. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian ovarian cancer study group. J Clin Oncol. 2012;30:2654-2663.
11. Pennington KP, Walsh T, Harrell MI, et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res. 2014;20:764-775.
12. Chao A, Chang TC, Lapke N, et al. Prevalence and clinical significance of BRCA1/2 germline and somatic mutations in Taiwanese patients with ovarian cancer. Oncotarget. 2016;7:85529-85541.
13. Cancer Genome Atlas Research N. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609-615.
14. Mafficini A, Simbolo M, Parisi A, et al. BRCA somatic and germline mutation detection in paraffin embedded ovarian cancers by next-generation sequencing. Oncotarget. 2016;7:1076-1083.
15. Bolton KL, Chenevix-Trench G, Goh C, et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA. 2012;307:382-390.
16. Tutt A, Ashworth A. The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. Trends Mol Med. 2002;8:571-576.
17. Zhao Q, Yang J, Li L, et al. Germline and somatic mutations in homologous recombination genes among Chinese ovarian cancer patients detected using next-generation sequencing. J Gynecol Oncol. 2017;28:e39.
18. Wu X, Wu L, Kong B, et al. The first nationwide multicenter prevalence study of germline BRCA1 and BRCA2 mutations in Chinese ovarian cancer patients. Int J Gynecol Cancer. 2017;27(8):1650-1657.
19. Kim YC, Zhao L, Zhang H, et al. Prevalence and spectrum of BRCA germline variants in mainland Chinese familial breast and ovarian cancer patients. Oncotarget. 2016;7:9600-9612.
20. Sakamoto I, Hirotsu Y, Nakagomi H, et al. BRCA1 and BRCA2 mutations in Japanese patients with ovarian, fallopian tube, and primary peritoneal cancer. *Cancer*. 2016;122:84-90.
21. Choi MC, Heo JH, Jang JH, et al. Germline mutations of BRCA1 and BRCA2 in Korean ovarian cancer patients: finding founder mutations. *Int J Gynecol Cancer*. 2015;25:1386-1391.
22. Lim MC, Kang S, Seo SS, et al. BRCA1 and BRCA2 germline mutations in Korean ovarian cancer patients. *J Cancer Res Clin Oncol*. 2009;135:1593-1599.
23. du Bois A, Floquet A, Kim JW, et al. Incorporation of pazopanib in maintenance therapy of ovarian cancer. *J Clin Oncol*. 2014;32:3374-3382.
24. Kim JW, Mahner S, Wu LY, et al. Pazopanib maintenance therapy in East Asian women with advanced epithelial ovarian cancer: results from AGO-OVAR16 and an East Asian study. *Int J Gynecol Cancer*. 2018;28:2-10.
25. Katsumata N, Yasuda M, Takahashi F, et al. Dose-dense paclitaxel once a week in combination with carboplatin every 3 weeks for advanced ovarian cancer: a phase 3, open-label, randomised controlled trial. *Lancet*. 2009;374:1331-1338.
26. Clamp AR, James EC, McNeil IA, et al. Weekly dose-dense chemotherapies in first-line epithelial ovarian, fallopian tube, or primary peritoneal carcinoma treatment (ICON8): primary progression free survival analysis results from a GCGIG phase 3 randomised controlled trial. *Lancet*. 2019;394:2084-2095.
27. Rustin GJ, Vergote I, Eisenhauer E, et al. Definitions for response and progression in ovarian cancer clinical trials incorporating RECIST 1.1 and CA 125 agreed by the gynecological cancer intergroup (GCIG). *Int J Gynecol Cancer*. 2011;21:419-427.
28. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38:e164.
29. Liu X, Jian X, Boerwinkle E, dbNSFP v2.0: a database of human non-synonymous SNVs and their functional predictions and annotations. *Hum Mutat*. 2013;34:E2393-E2402.
30. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405-424.
31. Stenson PD, Mort M, Ball EV, Shaw K, Phillips A, Cooper DN. The human gene mutation database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum Genet*. 2014;133:1-9.
32. Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014;42:D980-D985.
33. McLaughlin JR, Rosen B, Moody J, et al. Long-term ovarian cancer survival associated with mutation in BRCA1 or BRCA2. *J Natl Cancer Inst*. 2013;105:141-148.
34. Candido-dos-Reis FJ, Song H, Goode EL, et al. Germline mutation in BRCA1 or BRCA2 and ten-year survival for women diagnosed with epithelial ovarian cancer. *Clin Cancer Res*. 2015;21:652-657.
35. Cunningham JM, Cicek MS, Larson NB, et al. Clinical characteristics of ovarian cancer classified by BRCA1, BRCA2, and RAD51C status. *Sci Rep*. 2014;4:4026.
36. Tan DS, Rothermundt C, Thomas K, et al. “BRCAness” syndrome in ovarian cancer: a case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with BRCA1 and BRCA2 mutations. *J Clin Oncol*. 2008;26:5530-5536.
37. Ramus SJ, Gayther SA. The contribution of BRCA1 and BRCA2 to ovarian cancer. *Mol Oncol*. 2009;3:138-150.
38. Hennessy BT, Timms KM, Carey MS, et al. Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer. *J Clin Oncol*. 2010;28:3570-3576.
39. McAlpine JN, Porter H, Kobel M, et al. BRCA1 and BRCA2 mutations correlate with TP53 abnormalities and presence of immune cell infiltrates in ovarian high-grade serous carcinoma. *Mod Pathol*. 2012;25:740-750.
40. Dougherty BA, Lai Z, Hodgson DR, et al. Biological and clinical evidence for somatic mutations in BRCA1 and BRCA2 as predictive markers for olaparib response in high-grade serous ovarian cancers in the maintenance setting. *Oncotarget*. 2017;8:43653-43661.
41. Risch HA, McLaughlin JR, Cole DE, et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. *Am J Hum Genet*. 2001;68:700-710.
42. Pai T, Perrmuth-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. *Cancer*. 2005;104:2807-2816.
43. Hasmad HN, Lai KN, Wen WX, et al. Evaluation of germline BRCA1 and BRCA2 mutations in a multi-ethnic Asian cohort of ovarian cancer patients. *Gynecol Oncol*. 2016;141:318-322.
44. Rashid MU, Zaidi A, Torres D, et al. Prevalence of BRCA1 and BRCA2 mutations in Pakistani breast and ovarian cancer patients. *Int J Cancer*. 2006;119:2832-2839.
45. Johannesdottir G, Gudmundsdsson J, Bergthorsson JT, et al. High prevalence of the 999del5 mutation in Icelandic breast and ovarian cancer patients. *Cancer Res*. 1996;56:3663-3665.
46. Robles-Diaz L, Goldfrank DJ, Kauff ND, Robson M, Offit K. Hereditary ovarian cancer in Ashkenazi Jews. *Fam Cancer*. 2004;3:259-264.
47. Struwing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. *N Engl J Med*. 1997;336:1401-1408.
48. Kwon BS, Byun JM, Lee HJ, et al. Clinical and genetic characteristics of BRCA1/2 mutation in Korean ovarian cancer patients: a multicenter study and literature review. *Cancer Res Treat*. 2019;51:941-950.
49. Boyd J, Sonoda Y, Federici MG, et al. Clinicopathologic features of BRCA-linked and sporadic ovarian cancer. *JAMA*. 2000;283:2260-2265.
50. Malander S, Riddervheim M, Masback A, et al. One in 10 ovarian cancer patients carry germ line BRCA1 or BRCA2 mutations: results of a prospective study in Southern Sweden. *Eur J Cancer*. 2004;40:422-428.
51. Chetrit A, Hirsh-Yechezkel G, Ben-David Y, Lubin F, Friedman E, Sadetzki S. Effect of BRCA1/2 mutations on long-term survival of patients with invasive ovarian cancer: the national Israeli study of ovarian cancer. *J Clin Oncol*. 2008;26:20-25.
52. Yang D, Khan S, Sun Y, et al. Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. *JAMA*. 2011;306:1557-1565.
53. Mazoyer S. Genomic rearrangements in the BRCA1 and BRCA2 genes. *Hum Mutat*. 2005;25:415-422.
54. Park B, Dowty JG, Ahn C, et al. Breast cancer risk for Korean women with germline mutations in BRCA1 and BRCA2. *Breast Cancer Res Treat*. 2015;152:659-665.

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher's website.

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