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Original Article

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Germline and Somatic *BRCA1/2* Gene Mutational Status and Clinical Outcomes in Epithelial Peritoneal, Ovarian, and Fallopian Tube Cancer: Over a Decade of Experience in a Single Institution in Korea

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Purpose

This study aimed to present a single institutional experience with *BRCA1/2* gene tests and the effects of pathogenic mutations in epithelial peritoneal, ovarian, and fallopian tube cancer (POFTC) on survival outcomes.

Materials and Methods

We identified patients with epithelial POFTCs who underwent *BRCA1/2* gene testing by either germline or somatic methods between March 2007 and March 2020. Based on the *BRCA1/2* test results, patients were divided into *BRCA* mutation and wild-type groups, followed by comparisons of clinicopathologic characteristics and survival outcomes after primary treatment.

Results

The annual number of POFTC patients who received *BRCA1/2* gene tests increased gradually. In total, 511 patients were included and *BRCA1/2* mutations were observed in 143 (28.0%). Among 57 patients who received both germline and somatic tests, three (5.3%) showed discordant results from the two tests. Overall, no differences in progression-free survival (PFS; p=0.467) and overall survival (p=0.641) were observed between the *BRCA* mutation and wild-type groups; however, multivariate analyses identified *BRCA1/2* mutation as an independent favorable prognostic factor for PFS (adjusted hazard ratio [aHR], 0.765; 95% confidence interval [CI], 0.593 to 0.987; p=0.040). In 389 patients with International Federation of Gynecology and Obstetrics stage III-IV, different results were shown depending on primary treatment strategy: while *BRCA1/2* mutation significantly improved PFS in the subgroup of neoadjuvant chemotherapy (aHR, 0.619; 95% CI, 0.385 to 0.995; p=0.048), it did not affect patient PFS in the subgroup of primary debulking surgery (aHR, 0.759; 95% CI, 0.530 to 1.089; p=0.135).

Conclusion

BRCA1/2 mutations are frequently observed in patients with epithelial POFTCs, and such patients showed better PFS than did those harboring wild-type *BRCA1/2*.

Key words

Genital neoplasms, Female, Ovarian neoplasms, Germline test, Somatic test, *BRCA1/2* mutation, Clinical outcome, Survival outcome

Introduction

Ovarian cancer is the most lethal gynecologic malignancy and is estimated to account for 295,000 new cases and 185,000 cancer deaths annually worldwide [1]. Recent studies view epithelial peritoneal, ovarian, and fallopian tube cancers (POFTCs) as a single disease group that shares a common pathogenesis, diagnosis, and treatment [2]. Epithelial POFTCs tend to be diagnosed at an advanced-stage and show high recurrence and mortality rates, despite the standard primary treatment. Approximately 15% to 20% of patients with epithelial POFTCs present genetic predisposition or hereditary factors, with *BRCA1/2* identified as well-known causal genes [3,4].

Women harboring germline mutations in either *BRCA1/2* are at an excessive risk of developing both breast cancer (BC) and ovarian cancer [5,6]. Offspring of a germline *BRCA1/2*-mutation carrier have a 50% chance of inheriting the patho-

genic or likely pathogenic variant. Moreover, patients harboring germline or somatic *BRCA1/2* mutations with primary or platinum-sensitive relapsed POFTC experience positive survival outcomes from poly(ADP-ribose) polymerase (PARP) inhibitors based on their synthetic lethality [7-12]. Therefore, current guidelines from the Korean Society of Gynecologic Oncology recommend that patients with epithelial POFTC patients undergo *BRCA1/2* gene testing [13].

Previous studies have focused on the prognostic aspect of *BRCA1/2* mutations, frequently reporting that *BRCA1/2* mutations confer a survival advantage relative to wild-type *BRCA1/2* due to better response to platinum-based chemotherapy [14]. However, further analysis revealed that the study populations and designs, as well as the specific results, differ among studies. Although overall survival (OS) was improved in patients carrying *BRCA1/2* mutations [14,15], some studies identified advantages for only those harboring *BRCA2* mutations [16,17]. In our previous study that included patients with advanced-stage ovarian high-grade serous carcinoma (HGSC), longer progression-free survival (PFS) but not OS was associated with germline *BRCA1/2* mutations [18].

Therefore, additional scientific evidence concerning the effects of *BRCA1/2* mutations on POFTC prognosis according to the primary treatment strategy is necessary, especially in patients of Korean ethnicity. In this study, we investigated the impact of *BRCA1/2* mutational status on survival outcomes in patients with epithelial POFTC. Additionally, we present a single institutional experience with germline and somatic *BRCA1/2* gene testing not limited by initial International Federation of Gynecology and Obstetrics (FIGO) stage or histologic type.

Materials and Methods

1. Study population

Since starting germline *BRCA1/2* gene testing, our institution has conducted this test in patients with BC presenting a strong family history of BC or with family members harboring *BRCA1/2* mutations. In March 2007, patients with epithelial POFTC also began to receive germline *BRCA1/2* gene testing. In September 2017, our institutional hospital launched a targeted next-generation sequencing (NGS) cancer panel for clinical purposes, which enabled identification of somatic *BRCA1/2* mutational status in patients with epithelial POFTC.

To include all possible cases meeting the study purpose, we established the following inclusion criteria: (1) patients pathologically diagnosed with and treated for epithelial POFTC; and (2) patients who received either germline *BRCA1/2* gene testing or a somatic NGS cancer panel between March 2007 and March 2020, and thus whose germline or somatic *BRCA1/2* mutational status was verified. By contrast, we excluded patients with insufficient clinicopathologic data or those lost to follow-up during primary treatment.

We identified 563 patients from the Ovarian Cancer Cohort of the institution who met these criteria. For fair comparisons, we further excluded 52 patients who were enrolled in past or current clinical trials, during their primary treatment, which could affect survival outcomes.

2. Germline and somatic *BRCA1/2* gene test

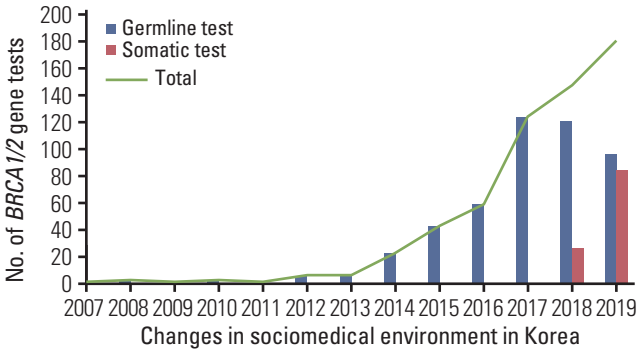
Germline *BRCA1/2* gene testing methods at the Seoul National University Hospital (SNUH) were described in our previous study [18]. As of February 2016, the method has been changed from direct sequencing (Sanger sequencing) to NGS of *BRCA1/2* genes. Sequence variants found in NGS were confirmed by Sanger sequencing.

For somatic *BRCA1/2* gene testing, we used an NGS cancer panel named "SNUH FIRST-Cancer panel version 3.1" and performed DNA collection and profiling from archival formalin-fixed paraffin-embedded (FFPE) tumor tissues, as described previously [19]. Briefly, genomic DNA was extracted from FFPE tissues using the ReliaPrep FFPE gDNA miniprep system (Promega, Madison, WI), and a library was constructed using the SureSelectXT target enrichment protocol (Agilent Technologies, Carlsbad, CA) for Illumina paired-end sequencing (2×101 bp), which was performed on the Illumina Hiseq 2500 platform (Illumina, Carlsbad, CA). Details of the reporting algorithms used for single-nucleotide variants, copy number variants, and structural variants were also described previously [19]. The SNUH FIRST-Cancer panel version 3.1 provides information on all exons of 183 genes, specific introns of 23 fusion genes, the *TERT* promoter region, eight microsatellite-instability markers, and 45 drug-target lesions, covering a total length of approximately 1.949 Mbp. Of these, we focused on genomic alterations of *BRCA1/2* genes.

We referenced the detected *BRCA1/2* variants in two representative databases, the Breast Cancer Information Core (BIC) and the National Institutes of Health open-access database of clinically observed variants and their classification (ClinVar), and the literature. Sequence variants in *BRCA1* and *BRCA2* were classified into five categories according to the recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [20]. In the present study, we regarded patients with "pathogenic" and "likely pathogenic" variants as the *BRCA* mutation group (*BRCA*mut; study group) and the rest of the patients as the *BRCA* wild-type group (*BRCA*wt; control group).

3. Data collection

Review of medical records and pathologic reports allowed collection of the following clinicopathologic data: age at diagnosis, histologic type, FIGO stage, initial serum cancer anti-



2012-04 NHIS covers *BRCA1/2* gene tests
2012-12 NHIS covers RRSO
2015-08 KFDA permits olaparib maintenance for *BRCA*mut, PSR, HGS POFTC (≥ 2 L)
2016-03 Positions statements on genetic test for POFTC, KSGO
2017-09 NHIS covers NGS cancer panel
2017-10 NHIS covers olaparib maintenance for *BRCA*mut, PSR, HGS POFTC (≥ 2 L)
2018-05 KFDA permits olaparib for *BRCA*mut, recurrent POFTC (≥ 4 L)
2019-03 KFDA permits niraparib maintenance for PSR, HGS POFTC (≥ 2 L)
2019-10 KFDA permits olaparib maintenance for PSR, HG POFTC (≥ 2 L)
2019-10 KFDA permits olaparib maintenance for *BRCA*mut, primary, HG POFTC (1 L)
2019-12 NHIS covers niraparib for germline *BRCA*mut, PSR, HGS POFTC (≥ 2 L)
2019-12 KFDA permits niraparib for *BRCA*mut or HRDpos, recurrent POFTC (≥ 4 L)

Fig. 1. Annual number of *BRCA1/2* gene tests among patients with peritoneal, ovarian, and fallopian tube cancers (POFTCs) and according to changes in sociomedical environment in Korea. NHIS, National Health Insurance Service; RRSO, risk reducing salpingo-oophorectomy; KFDA, Korea Food and Drug Administration; PSR, platinum-sensitive relapsed; HGS, high-grade serous; KSGO, Korean Society of Gynecologic Oncology; NGS, next-generation sequencing; HG, high-grade; HRDpos, homologous recombination deficiency-positive.

gen 125 levels, and primary treatment strategy. We considered optimal debulking to have occurred when the surgery resulted in the largest size of the residual tumor being < 1 cm. All patients received taxane- and platinum-based chemotherapy as part of their primary treatment unless they had low-grade IA/IB disease according to the 2014 FIGO staging system. Additionally, we retrieved personal and familial histories of cancer and the number of affected family members up to the second degree.

For survival analyses, PFS was defined as the time interval between the date of initial diagnosis and the date of disease progression confirmed by the Response Evaluation Criteria in Solid Tumours ver. 1.1 [21]. OS was defined as the time interval between the date of initial diagnosis to the date of cancer-related death or last visit.

4. Statistical analysis

Baseline clinicopathologic characteristics and survival outcomes were compared between the *BRCA*mut and *BRCA*wt groups. We used a Student's t or Mann-Whitney U test for comparisons of continuous variables, and Pearson's chi-squared or Fisher exact test for comparisons of categorical variables. For survival analyses, the Kaplan-Meier method with log-rank test and Cox proportional hazards regression models were used. We calculated the adjusted hazard ratio

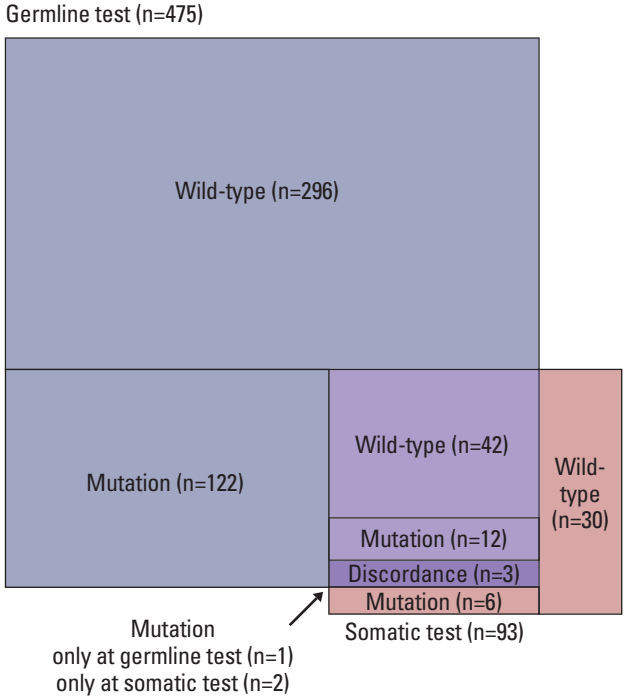


Fig. 2. Aerial chart depicting proportion of patients who underwent germline and somatic *BRCA1/2* gene tests along with the test results.

Table 1. Clinicopathologic characteristics of the study population

Characteristic	Total (n=511)	*BRCA* wild-type (n=368)	*BRCA* mutation (n=143)	p-value
Age (yr)	54.3±10.9	54.5±11.5	53.9±9.3	0.557
Parity	1.9±1.3	1.9±1.3	1.9±1.0	0.394
Origin				
Ovary	481 (94.1)	345 (93.8)	136 (95.1)	0.705
Fallopian tube	14 (2.7)	10 (2.7)	4 (2.8)	
Peritoneum	16 (3.1)	13 (3.5)	3 (2.1)	
Hx of BC	58 (11.4)	29 (7.9)	29 (20.3)	< 0.001
Hx of other cancers	28 (5.5)	18 (4.9)	10 (7.0)	0.349
Family Hx of POFTC	27 (5.3)	7 (1.9)	20 (14.0)	< 0.001
No. of relatives	0.1±0.2	0.0±0.1	0.2±0.4	< 0.001
Family Hx of BC	45 (8.8)	16 (4.3)	29 (20.3)	< 0.001
No. of relatives	0.1±0.4	0.1±0.2	0.3±0.6	< 0.001
Family Hx of other cancers	111 (21.7)	74 (20.1)	37 (25.9)	0.156
FIGO stage				
I	72 (14.1)	65 (17.7)	7 (4.9)	0.001
II	50 (9.8)	39 (10.6)	11 (7.7)	
III	259 (50.7)	177 (48.1)	82 (57.3)	
IV	130 (25.4)	87 (23.6)	43 (30.1)	
Histology				
High-grade serous	368 (72.0)	246 (66.8)	122 (85.3)	0.001
Low-grade serous	11 (2.2)	10 (2.7)	1 (0.7)	
Endometrioid	43 (8.4)	37 (10.1)	6 (4.2)	
Mucinous	16 (3.1)	13 (3.5)	3 (2.1)	
Clear cell	42 (8.2)	40 (10.9)	2 (1.4)	
Mixed	14 (2.7)	10 (2.7)	4 (2.8)	
Others	8 (1.6)	6 (1.6)	2 (1.4)	
Unknown	9 (1.8)	6 (1.6)	3 (2.1)	
Tumor grade				
1	30 (5.9)	27 (7.3)	3 (2.1)	0.027
2	28 (5.5)	23 (6.3)	5 (3.5)	
3	438 (85.7)	306 (83.2)	132 (92.3)	
Unknown	15 (2.9)	12 (3.3)	3 (2.1)	
CA-125 (IU/mL)	695.5 (3.4-17,313)	666.5 (3.4-15,700)	767.0 (5.1-17,313)	0.085
Primary treatment strategy				
PDS	379 (74.2)	278 (75.5)	101 (70.6)	0.255
NAC	132 (25.8)	90 (24.5)	42 (29.4)	
Residual tumor after PDS/IDS[a]				
No gross	374 (73.2)	278 (75.5)	96 (67.1)	0.224
< 1 cm	70 (13.7)	48 (13.0)	22 (15.4)	
1-2 cm	23 (4.5)	13 (3.5)	10 (7.0)	
≥ 2 cm	22 (4.3)	17 (4.6)	5 (3.5)	
Unknown	13 (2.5)	6 (1.6)	7 (4.9)	
Chemotherapy at primary treatment				
Bevacizumab- containing regimen	30 (5.9)	19 (5.2)	11 (7.7)	0.362
Non-bevacizumab regimen	464 (90.8)	335 (91.0)	129 (90.2)	
No chemotherapy	17 (3.3)	14 (3.8)	3 (2.1)	
Recurrence[b]	324 (63.4)	231 (62.8)	93 (65.0)	0.634
PSR[c]	238 (46.6)	156 (42.4)	82 (57.3)	0.001
PRR	78 (15.3)	67 (18.2)	11 (7.7)	

(Continued to the next page)

Table 1. Clinicopathologic characteristics of the study population

Characteristic	Total (n=511)	*BRCA* wild-type (n=368)	*BRCA* mutation (n=143)	p-value
Genetic test methods				
Germline only	418 (81.8)	296 (80.4)	122 (85.3)	0.264
Somatic only	36 (7.0)	30 (8.2)	6 (4.2)	
Both	57 (11.2)	42 (11.4)	15 (10.5)	
***BRCA1* mutational status**				
Wild-type	409 (80.0)	368 (100)	41 (28.7)	< 0.001
Mutation	102 (20.0)	0	102 (71.3)	
***BRCA2* mutational status**				
Wild-type	469 (91.8)	368 (100)	101 (70.6)	< 0.001
Mutation	42 (8.2)	0	42 (29.4)	

Values are presented as mean±SD, number (%), or median (range). Hx, history; BC, breast cancer; POFTC, peritoneal, ovarian, and fallopian tubal cancers; FIGO, International Federation of Gynecology and Obstetrics; CA-125, cancer antigen 125; PDS, primary debulking surgery; NAC, neoadjuvant chemotherapy; IDS, interval debulking surgery; PSR, platinum-sensitive recurrence; PRR, platinum-resistant recurrence; SD, standard deviation. [a]Nine patients did not receive debulking surgery, [b]Among the recurred, eight patients did not receive taxane- and platinum-based chemotherapy before, [c]PSR was defined as relapse ≥ 6 months after completion of taxane- and platinum-based chemotherapy, whereas PRR as relapse < 6 months.

(aHR) and 95% confidence interval (CI) for each variable. All statistical analyses were conducted by using SPSS software ver. 25.0 (IBM Corp., Armonk, NY), and a $p < 0.05$ was regarded as statistically significant.

5. Ethical statement

This retrospective cohort study was approved by the Institutional Review Board of SNUH (No. C-2005-042-1122) and performed in accordance with the principles of the Declaration of Helsinki. The requirement for informed consent was waived.

Results

1. *BRCA1/2* gene test results

The annual number of POFTC patients who received *BRCA1/2* gene tests increased gradually according to a series of sociomedical environment changes in Korea (Fig. 1). Of 511 patients who underwent *BRCA1/2* gene tests (418, 36, and 57 for germline test only, somatic test only, and both tests, respectively), *BRCA1/2* mutations were observed in 143 (28.0%), with 20.0% and 8.2% of patients harboring *BRCA1* and *BRCA2* mutations, respectively. One patient harbored mutations in both genes; however, germline testing identified only a *BRCA2* mutation (c.9097dupA), whereas somatic testing identified an additional *BRCA1* mutation (c.2206_2207delGA).

We observed differential *BRCA1/2* mutational status in patients with POFTC according to the presence of BC and/or other cancers, such as colorectal and gastric cancers (S1 Fig.). Although the prevalence of *BRCA1/2* mutations was lowest among patients presenting POFTC only (24.9%), it was highest among those presenting POFTC, BC, and another cancer (triple cancers; 75.0%). Of the 54 patients presenting both POFTC and BC, *BRCA1/2* mutations were identified in 26 (48.1%).

Among 57 patients who received both germline and somatic tests, three (5.3%) showed discordant results in their classification into the *BRCA*mut and *BRCA*wt groups (Fig. 2). Specifically, one patient harboring germline *BRCA1* mutation showed restoration of a wild-type *BRCA1* sequence according to somatic testing (true reversion), and the other two with germline *BRCA1/2* wild-type were identified as harboring somatic *BRCA1* mutation (acquired mutation). Details of *BRCA1/2* test results and clinical information of the 57 patients are presented in S2 Table.

2. Characteristics of the study population

Patient characteristics are shown in Table 1. Age at diagnosis of POFTC was similar between the *BRCA*mut and *BRCA*wt groups. However, patients with *BRCA* mutations displayed significantly higher personal and family histories of BC and a higher family history of POFTC relative to those without *BRCA* mutations. Initial disease presentation also differed between groups, with the *BRCA*mut group showing more advanced disease and more frequent HGSC histology. In terms of primary treatment, there were no differences in the proportion of neoadjuvant chemotherapy (NAC) cases and residual tumor after debulking surgery between groups. In this study, 5.9% (30/511) of the study population received be acizumab-containing chemotherapy during primary treatment, and the proportion of bevacizumab users was similar between the *BRCA*mut and *BRCA*wt groups. No patient received maintenance with a PARP inhibitor after primary treatment.

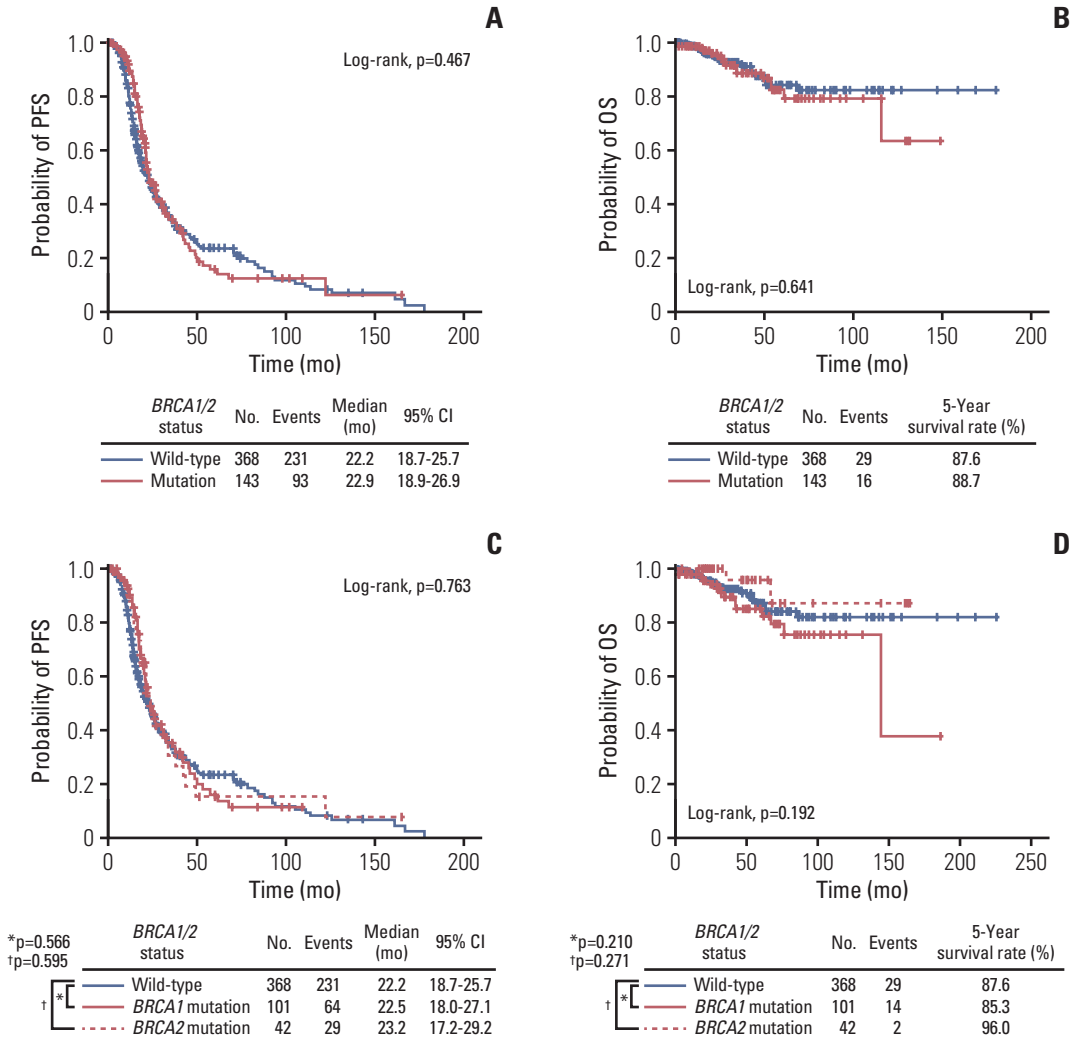


Fig. 3. Survival outcomes of the study population (A, B), and further comparisons according to the mutated *BRCA* gene (C, D). (A, C) Progression-free survival (PFS). (B, D) Overall survival (OS).

BRCA1/2 mutations were observed in 33.2% of patients with HGSC (n=368), a higher percentage than in the whole study population. As shown in S3 Table, patient characteristics were similar between the *BRCA*mut and *BRCA*wt groups, except for patient age, personal history of BC, and family history of BC and POFTC. In patients who had histologic types other than HGSC (non-HGSC, n=134), incidence of *BRCA1/2* mutation was 13.4%. As shown in S4 Table, patient characteristics, such as primary treatment strategy and residual tumor after debulking surgery, were similar between the two groups, whereas family history of BC and POFTC differed.

3. Clinical outcomes of all study populations

During the median observation period of 42.8 months, 93 patients (65.0%) in the *BRCA*mut group and 231 (62.8%) in the *BRCA*wt group experienced disease recurrence. Despite the higher proportion of platinum-sensitive recurrence in the *BRCA*mut group (p=0.001), which referred to recurrence within 6 months after completion of platinum-based primary treatment, the two groups showed similar PFS (median, 22.9 vs. 22.2 months; p=0.467) (Fig. 3A). However, multivariate analyses adjusting for age, FIGO stage, histologic type, primary treatment strategy, and residual tumor after debulking surgery revealed *BRCA1/2* mutation as an independent favorable prognostic factor for PFS (aHR, 0.765; 95% CI, 0.593 to 0.987; p=0.040) (Table 2). Both *BRCA*mut and *BRCA*wt groups showed similar OS (5-year survival rate, 88.7% vs. 87.6%; p=0.641) (Fig. 3B), and multivariate analyses revealed that presence of *BRCA1/2* mutations did not affect patient OS (Table 2). Use of bevacizumab in primary treatment did not improve patient PFS and OS in univariate and multivariate analyses.

Regarding the specific genes with mutations, we subdivided the *BRCA*mut group into the *BRCA1*mut (n=101) and

Table 2. Factors associated with survival outcomes

Characteristic	Progression-free survival						Overall survival					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	aHR	95% CI	p-value	HR	95% CI	p-value	aHR	95% CI	p-value
Age (yr)												
< 55	1			1			1			1		
≥ 55	1.590	1.273-1.985	< 0.001	1.325	1.043-1.683	0.021	2.237	1.218-4.107	0.009	1.704	0.904-3.211	0.099
FIGO stage												
I-II	1			1			1			1		
III	2.435	1.746-3.397	< 0.001	2.296	1.563-3.371	< 0.001	1.502	0.613-3.678	0.374	1.078	0.378-3.079	0.888
IV	3.568	2.478-5.139	< 0.001	2.637	1.681-4.138	< 0.001	2.768	1.068-7.176	0.036	1.490	0.465-4.775	0.502
Histology												
High-grade serous	1			1			1			1		
Non–high-grade serous	0.620	0.472-0.813	0.001	1.085	0.795-1.483	0.607	0.803	0.396-1.625	0.541	1.219	0.545-2.724	0.630
Primary treatment strategy												
PDS	1			1			1			1		
NAC	2.103	1.657-2.670	< 0.001	1.511	1.131-2.019	0.005	2.071	1.117-3.837	0.021	2.003	0.969-4.142	0.061
Residual tumor after PDS/IDS												
< 1 cm	1			1			1			1		
≥ 1 cm	1.738	1.229-2.457	0.002	1.310	0.894-1.918	0.166	2.729	1.381-5.395	0.004	2.676	1.261-5.678	0.010
***BRCA* mutational status**												
Wild-type	1			1			1			1		
Mutation	0.914	0.718-1.164	0.467	0.765	0.593-0.987	0.040	1.156	0.627-2.131	0.642	1.163	0.623-2.171	0.636

HR, hazard ratio; CI, confidence interval; aHR, adjusted hazard ratio; FIGO, International Federation of Gynecology and Obstetrics; PDS, primary debulking surgery; NAC, neoadjuvant chemotherapy; IDS, interval debulking surgery.

Table 3. Factors associated with survival outcomes in patients with FIGO stage III to IV disease

Characteristic	Progression-free survival						Overall survival					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	aHR	95% CI	p-value	HR	95% CI	p-value	aHR	95% CI	p-value
Age (yr)												
< 55	1			1			1			1		
≥ 55	1.431	1.127-1.815	0.003	1.406	1.076-1.837	0.013	2.261	1.160-4.405	0.017	1.650	0.831-3.276	0.152
FIGO stage												
III	1			1			1			1		
V	1.476	1.148-1.897	0.002	1.184	0.872-1.607	0.279	1.894	0.979-3.665	0.058	1.294	0.625-2.681	0.487
Histology												
High-grade serous	1			1			1			1		
Non–high-grade serous	1.068	0.764-1.492	0.701	1.345	0.931-1.942	0.114	1.029	0.430-2.463	0.949	1.250	0.466-3.355	0.658
Initial serum CA-125 (IU/mL)												
< 900	1			1			1			1		
≥ 900	1.294	1.006-1.666	0.045	1.163	0.887-1.525	0.275	1.842	0.952-3.566	0.070	1.456	0.723-2.933	0.293
Primary treatment strategy												
PDS	1			1			1			1		
NAC	1.683	1.315-2.153	< 0.001	1.502	1.093-2.066	0.012	1.918	1.005-3.664	0.048	2.105	0.963-4.604	0.062
Residual tumor after PDS/IDS												
< 1 cm	1			1			1			1		
≥ 1 cm	1.435	1.010-2.038	0.044	1.596	1.078-2.364	0.020	2.438	1.210-4.913	0.013	2.769	1.258-6.095	0.011
***BRCA* mutational status**												
Wild-type	1			1			1			1		
Mutation	0.828	0.642-1.068	0.147	0.722	0.546-0.956	0.023	0.986	0.512-1.899	0.967	1.066	0.547-2.078	0.851

FIGO, International Federation of Gynecology and Obstetrics; HR, hazard ratio; CI, confidence interval; aHR, adjusted hazard ratio; CA-125, cancer antigen 125; PDS, primary debulking surgery; NAC, neoadjuvant chemotherapy; IDS, interval debulking surgery.

Table 4. Factors associated with progression-free survival in patient with FIGO stage III to IV disease according to primary treatment strategy

Characteristic	Primary debulking surgery						Neoadjuvant chemotherapy					
	Univariate analysis			Multivariate analysis			Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	aHR	95% CI	p-value	HR	95% CI	p-value	aHR	95% CI	p-value
Age (yr)												
< 55	1			1			1			1		
≥ 55	1.286	0.952-1.737	0.101	1.491	1.060-2.097	0.022	1.543	1.020-2.333	0.040	1.508	0.948-2.401	0.083
FIGO stage												
III	1			1			1			1		
IV	1.587	1.107-2.275	0.012	1.873	1.263-2.777	0.002	0.895	0.604-1.327	0.581	0.691	0.439-1.088	0.111
Histology												
High-grade serous	1			1			1			1		
Non–high-grade serous	1.203	0.815-1.775	0.352	1.658	1.085-2.533	0.019	0.856	0.423-1.736	0.667	1.076	0.458-2.529	0.866
Initial serum CA-125 (IU/mL)												
< 900	1			1			1			1		
≥ 900	1.201	0.871-1.658	0.264	1.189	0.848-1.668	0.316	1.219	0.787-1.889	0.374	1.256	0.786-2.007	0.341
Residual tumor after PDS/IDS												
< 1 cm	1			1			1			1		
≥ 1 cm	1.471	0.975-2.218	0.066	1.340	0.857-2.096	0.200	1.887	0.943-3.775	0.073	3.200	1.357-7.544	0.008
***BRCA* mutational status**												
Wild-type	1			1			1			1		
Mutation	0.940	0.682-1.295	0.705	0.759	0.530-1.089	0.135	0.659	0.431-1.008	0.054	0.619	0.385-0.995	0.048

FIGO, International Federation of Gynecology and Obstetrics; HR, hazard ratio; CI, confidence interval; aHR, adjusted hazard ratio; CA-125, cancer antigen 125; PDS, primary debulking surgery; IDS, interval debulking surgery.

*BRCA2*mut (n=42) groups. The one patient harboring mutations in both genes was placed into the *BRCA2*mut group for statistical purposes. The *BRCA1*mut and *BRCA2*mut groups showed similar PFS and OS relative to the *BRCA*wt group (Fig. 3C and D). In multivariate analyses, *BRCA1* mutation rather than *BRCA1/2* wild-type was not a prognostic factor for improved PFS (aHR, 0.773; 95% CI, 0.575 to 1.040; p=0.089) and OS (aHR, 1.689; 95% CI, 0.870 to 3.280; p=0.121). Additionally, *BRCA2* mutation did not affect patient PFS (aHR, 0.780; 95% CI, 0.522 to 1.166; p=0.226) and OS (aHR, 0.403; 95% CI, 0.095 to 1.703; p=0.216), compared to *BRCA1/2* wild-type.

4. Subgroup analysis according to histologic type

We performed subgroup analyses of patients in order to investigate the effect of *BRCA1/2* mutations on survival outcomes according to the histologic type. Among patients with HGSC (n=368), no differences in PFS (p=0.576) and OS (p=0.980) were observed between the *BRCA*mut and *BRCA*wt groups (S5A and S5B Fig.). In multivariate analyses, *BRCA1/2* mutation was not associated with patient PFS (aHR, 0.785; 95% CI, 0.586 to 1.051; p=0.104) (S6 Table).

Among patients with non-HGSC (n=134), the *BRCA*mut and *BRCA*wt groups showed similar PFS (p=0.321) and OS (p=0.450) (S5C and S5D Fig.). Multivariate analyses revealed that presence of *BRCA1/2* mutations did not affect patient PFS (aHR, 0.530; 95% CI, 0.252 to 1.115; p=0.094) (S6 Table).

5. Subgroup analysis according to primary treatment strategy

We then performed subgroup analyses of only patients with stage III to IV disease (n=389) in order to determine differences in the effect of *BRCA1/2* mutations on survival outcomes according to the primary treatment strategy. Overall, the *BRCA*mut and *BRCA*wt groups showed similar PFS (p=0.146) and OS (p=0.967) (S7A-S7C Fig.). However, multivariate analyses identified *BRCA1/2* mutation as an independent favorable prognostic factor for PFS (aHR, 0.722; 95% CI, 0.546 to 0.956; p=0.023), although not for OS (aHR, 1.066; 95% CI, 0.547 to 2.078; p=0.851) (Table 3).

Among patients with stage III to IV disease who underwent primary debulking surgery (n=257), we observed no differences in PFS (p=0.705) or OS (p=0.768) between the *BRCA*mut and *BRCA*wt groups and no difference in PFS according to specific gene mutation (S7D-S7F Fig.). Multivariate analyses revealed that *BRCA1/2* mutation did not affect patient PFS (aHR, 0.759; 95% CI, 0.530-1.089; p=0.135) (Table 4).

Among patients with stage III to IV disease who underwent NAC (n=132), the *BRCA*mut group showed better PFS with marginal significance than did the *BRCA*wt group (p=0.052), whereas a similar OS was observed between the two groups (p=0.619) (S7G-S7I Fig.). Additionally, multivariate analyses identified *BRCA1/2* mutation as an independent favorable factor for improved PFS (aHR, 0.619; 95% CI, 0.385 to 0.995; p=0.048) (Table 4).

Discussion

In this single-institution, retrospective cohort study, we presented the *BRCA1/2* mutational status of patients with epithelial POFTC and evaluated its effect on survival outcomes. We found a high incidence (28.0%) of *BRCA1/2* mutation and that germline or somatic *BRCA1/2* mutations were associated with better PFS than were wild-type *BRCA* genes.

Identification of patients with *BRCA1/2* mutations and evaluation of their clinical outcomes are important issues in POFTC. Individuals with POFTC confirmed as harboring germline *BRCA1/2* mutations have an opportunity to undergo treatment with PARP inhibitors. At the same time, they should undergo cancer surveillance for BC or other *BRCA*-related cancers. Additionally, their family members might benefit from *BRCA1/2* gene testing in aspect of cancer prevention.

The incidence of *BRCA1/2* mutation in patients with POFTCs varies among different histologic types, with HGSC being the most common type and showing the highest mutation incidence (20%-25%) [22-24]. Consistently with previous studies, we found that the incidence of *BRCA1/2* mutations was higher in patients with HGSC (33.2%) and lower in non-HGSC patients (13.4%) relative to the overall study population (28.0%). Specifically, incidences of *BRCA1/2* mutations in endometrioid and clear cell carcinomas were 14.0% (6/43) and 4.8% (2/42), respectively. In Canadian and Australian populations, previous studies have reported that germline *BRCA1/2* mutations were found in approximately 7% to 8% of patients with ovarian endometrioid and clear cell carcinoma [15,25]. Although our study included a substantial number of Korean patients with non-HGSC POFTC (n=134), the sample size for each histologic type was so small that proper comparisons were difficult between our study results and those from previous studies. Considering that ovarian clear cell carcinoma is more common in East Asian populations than in Western populations [26], *BRCA1/2* test results from East Asians might differ from those from other regions. Therefore, an East Asian collaborative research is necessary to ascertain the exact incidences of *BRCA1/2* mutations in specific histologic types of epithelial POFTC.

Regarding survival outcomes, we identified *BRCA1/2* mutation as a favorable prognostic factor for PFS in the entire study population in consistence with previous studies reporting associations between *BRCA1/2* mutation and improved PFS [14,15,18,27]. We also observed similar results in patients with stage III to IV disease, especially in those who underwent NAC. This improved PFS in patients with POFTC har-

boring *BRCA1/2* mutations is likely due to a high response rate to platinum-based chemotherapy mediated by vulnerability to DNA double-strand breaks [28,29]. However, *BRCA1/2* mutational status did not affect patient PFS in the subgroup of primary debulking surgery, which might be explained by our institution's high optimal debulking rate (85.8%; 211/246), possibly offsetting *BRCA*-related favorable chemotherapy response.

Despite the elongated PFS in patients with *BRCA1/2* mutations, we did not observe differences in patient OS according to *BRCA1/2* mutational status, which differs from previous studies [15,30,31]. This deviation might originate from our study population not being limited by a specific stage or histologic type of epithelial POFTCs. In addition, as *BRCA* mutated tumor gains resistance through the sequential chemotherapy, it is likely that the initial high response to chemotherapy does not lead to improved OS. Although the mechanisms of acquired chemoresistance are heterogeneous, researchers have commonly reported secondary mutations in *BRCA1/2* genes, or reversion mutations, that restores homologous recombination repair functions [32,33]. Sokolenko et al. [34] also reported rapid selection of pre-existing *BRCA1*-proficient tumor clones during chemotherapy in ovarian cancer patients who had germline *BRCA1* mutations. Development of individualized, novel treatment strategies reflecting each patient's specific mechanisms underlying chemoresistance are highly warranted to improve patient OS.

The advent of treatment strategies involving the two PARP inhibitors olaparib and niraparib for POFTC has increased the demand for *BRCA1/2* gene testing in Korea. Based on the findings that tumors with somatically acquired *BRCA1* or *BRCA2* pathogenic mutations respond to PARP inhibitors [10-12], physicians at our institution are recommending somatic testing to patients harboring wild-type *BRCA1/2* according to germline test results and vice versa in order to expand candidate options for PARP inhibitors. As a result, 57 patients from the study population received both germline and somatic tests. The results of both tests within the same patient can be inconsistent due to differences in both the methods and specimens used. In the present study, among 13 patients with both germline and somatic *BRCA1/2* mutations, two (15.4%) showed different variations of *BRCA1/2* mutations, which is similar to a previous study from another institution in Korea [35]. However, a difference in patient classification represents an important issue. Classification of patients into *BRCA*mut and *BRCA*wt groups resulted in a 5.3% (3/57) discordance rate. Of the 57 patients receiving both germline and somatic tests, solitary germline testing failed to identify two patients harboring somatic *BRCA1/2* mutations (3.5%), and solitary somatic testing failed to identify one patient harboring germline *BRCA1/2* mutations (1.8%). Therefore, this suggests an advantage to conducting both germline and somatic testing in order to identify single *BRCA1/2* mutations. However, clinicians need to consider the accuracy of each test, as well as testing cost-effectiveness and available resources.

Although the Korea Food and Drug Administration (KFDA) recently permitted olaparib maintenance for newly diagnosed, high-grade POFTC involving *BRCA1/2* mutation in October 2019, few patients at our institution have actually received olaparib in this setting due to its high price; in the current study, none of the patients received maintenance with olaparib after primary treatment. Additionally, the use of niraparib for first-line maintenance has not yet been permitted by the KFDA. Therefore, we could not observe the substantial survival benefit from PARP inhibitors reported in the phase 3 SOLO-1 [7] or PRIMA [8] trials in this study. It is expected that more patients will use PARP inhibitors in a primary setting if the price of the drugs is lowered or if changes in the sociomedical environment encourage the use of such drugs. However, as PARP inhibitors continue to increase in popularity, further investigation of the exclusive effect of *BRCA1/2* mutations on survival outcomes will be increasingly difficult to conduct.

This study has several limitations. First, selection bias or survival bias might exist due to the retrospective study design. Especially, in terms of baseline characteristics, FIGO stage differed significantly between the *BRCA* mutation and wild-type groups. Second, initial tumor load and disease patterns were not examined. Third, despite collecting cases of *BRCA1/2* gene tests over a considerable time period (e.g., > 10 years for the germline test), some might argue that the sample size was small, especially for further comparisons according to the mutated *BRCA* gene types. Fourth, we only investigated details of the primary treatment. Nevertheless, because very small portion (5.9%) of the study population received bevacizumab during primary treatment, we could not assess survival benefit from bevacizumab exactly in relation with the *BRCA1/2* mutational status. Finally, although we recognize that somatic testing conducted using an NGS cancer panel reports variants of genes other than *BRCA1/2*, we only considered and collected *BRCA1/2* results for study purposes. Currently, we are planning further studies to investigate associations between deficiency in homologous recombination repair genes other than *BRCA1/2* and POFTC patient survival outcomes. Nevertheless, we attempted to organize the experiences of our institution regarding *BRCA1/2* gene testing and present them with systematic survival analyses.

In conclusion, we found that *BRCA1/2* mutations were frequently observed in patients with epithelial POFTCs. This study demonstrated that patients harboring pathogenic *BRCA1/2* mutations showed a better prognosis with longer PFS than did those harboring wild-type *BRCA1/2*. These findings might have important implications for real-world practice and clinical trial design.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (https://www.e-crt.org).

Conflicts of Interest

Conflicts of interest relevant to this article was not reported.

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