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Prevalence and oncologic outcomes of *BRCA1/2* mutation and variant of unknown significance in epithelial ovarian carcinoma patients in Korea

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Objective

BRCA mutational status is important in the management of ovarian cancer, but there is a lack of evidence supporting genetic testing in Asian populations. This study was performed to investigate the prevalence and prognostic outcomes of *BRCA1/2* mutation and variant of unknown significance (VUS) in Korean patients diagnosed with epithelial ovarian cancer (EOC).

Methods

Among patients newly diagnosed with EOC between January 2007 and January 2017, those tested for germline *BRCA1/2* mutation were studied, regardless of family history. Overall survival (OS) and progression-free survival (PFS) were compared between the patients with and without *BRCA1/2* mutation and VUS.

Results

A total of 313 patients underwent *BRCA* testing: 88 patients had a *BRCA1/2* mutation and 48 patients had a *BRCA1/2* VUS (28.1% and 15.3%, respectively). There were no significant associations between *BRCA1/2* mutation, *BRCA1/2* wild-type, or *BRCA1/2* VUS with age at diagnosis, histologic distribution, or residual disease status after primary cytoreductive surgery. *BRCA1* mutation, including *BRCA1* VUS, showed no difference in PFS or OS compared to *BRCA1* wild-type. In contrast, *BRCA2* mutation showed longer PFS than that of *BRCA2* wild-type (P=0.04) or *BRCA2* VUS (P=0.02). *BRCA2* mutation, including *BRCA2* VUS, did not show any difference in OS compared to *BRCA2* wild-type.

Conclusion

BRCA mutation and *BRCA* VUS had similar clinical characteristics and survival outcomes, except that *BRCA2* mutation showed better PFS. The results of this study will help to understand the prognostic significance of *BRCA* mutation and VUS in Korean patients.

Keywords: *BRCA1* gene; *BRCA2* gene; Variant of unknown significance; Epithelial ovarian cancer

Introduction

Epithelial ovarian cancer (EOC) is the second most common gynecologic cancer worldwide, and most cases (75–80%) are found in the advanced stage at the time of diagnosis. Even with the therapeutic advances of maximal debulking surgery and platinum-based chemotherapy, the survival rate in EOC remains poor. The overall 5-year survival rate was only approximately 40% following surgical and systemic chemotherapy treatments [1]. Recently, poly (ADP-ribose) polymerase inhibitors (PARPi) have been widely used in EOC

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patients carrying *BRCA* mutation, and information regarding *BRCA1/2* mutations tends to be more important in the treatment of EOC.

BRCA1 and *BRCA2* have significant roles in DNA repair mechanisms, and proteins encoded by the *BRCA* genes are necessary for homologous recombination-mediated DNA repair of double-strand breaks, which subsequently maintains DNA stability and prevents uncontrolled cell growth [2]. Interestingly, improved survival outcomes were reported in EOC patients with *BRCA1/2* mutation compared to those with wild-type *BRCA*. Chemotherapy containing platinum, which is a cytotoxic agent causing DNA damage, shows a high response rate with improved survival in germline *BRCA1/2* mutation carriers compared to sporadic ovarian cancers [3,4].

Although the status of *BRCA1/2* mutations is important in clinical decision making and prognostication of EOC, there have been only a few studies with small sample sizes conducted in Asian populations including Korean patients. Some ovarian cancer studies with germline *BRCA1/2* mutation have been analyzed in Korea, and 33% of patients with *BRCA1/2* mutation had a family history of EOC. Patients with *BRCA1/2* mutation had a longer overall survival (OS) than those with *BRCA1/2* wild-type in Korea [5-7]. Another study showed that in advanced-stage high-grade serous ovarian cancer, patients with *BRCA1/2* mutation had a longer progression-free survival (PFS) than those with *BRCA* wild-type [8].

In addition, the clinical characteristics or prognosis of EOC patients who have *BRCA1/2* variant of unknown significance (VUS) have not been analyzed extensively, especially in Asian patients. A VUS is an identified DNA alteration not known to be deleterious and with unknown effects on protein function [9]. Patients diagnosed with *BRCA1/2* VUS typically receive simple counselling and observation [7,10].

Therefore, the purpose of this study is to analyze the prevalence of and survival outcomes according to *BRCA1/2* mutation status, including *BRCA* VUS, in Korean patients with EOC.

Materials and methods

1. Patients and study design

This was a retrospective cohort study performed in a single institution in Korea. Patients newly diagnosed with EOC between January 2007 and January 2017 were analyzed. Eligible patients included women who were newly diagnosed with EOC, either fallopian tube or primary peritoneal carcinoma; were rated as International Federation of Gynecology and Obstetrics (FIGO) stage I to IV; and underwent genetic *BRCA* testing. Women with high-grade serous carcinoma and those with mucinous, clear cell, low-grade serous or endometrioid, mixed epithelial adenocarcinoma, or undifferentiated carcinoma were included. The study was approved by Samsung Medical Center Institutional Review Board (IRB No. 2019-05-080-001).

2. *BRCA1/2* mutation analysis

Genomic DNA was extracted from ethylenediaminetetraacetic acid-anticoagulated whole blood using the Wizard® Genomic DNA Purification Kit according to the manufacturer's instructions (Promega, Madison, WI, USA). Full sequencing of all coding exons and all adjacent exon/intron boundaries of *BRCA1/2* was achieved using the Ion AmpliSeq™ *BRCA1* and *BRCA2* Panel (Life Technologies, Carlsbad, CA, USA) containing 167 primer pairs, and Ion AmpliSeq kit 2.0. The amplicons were clonally amplified through emulsion PCR using the IT OneTouch Template Kit 2.0 on an IT OneTouch system (Life Technologies) following the manufacturer's instructions. Targeted sequencing was performed using the Ion PGM platform with the Ion PGM sequencing 200 kit, following the manufacturer's instructions.

3. Treatment and follow-up

Patients underwent primary surgery followed by platinum-based combination chemotherapy or were treated with neoadjuvant chemotherapy followed by interval debulking surgery. After treatment, patients were followed up every 3 months for the first 2 years and then every 6 months for up to 5 years. Patients were monitored on the basis of clinical, biochemical, and imaging examinations. All clinical and pathologic data were collected through electronic chart review, including age at diagnosis, cancer antigen (CA)-125 level, residual disease status after primary cytoreductive surgery, histologic type, tumor grade, and FIGO stage. Surgical outcomes were categorized as either optimal if residual tumor size was less than 1 centimeter or suboptimal if it was the same or greater than 1 centimeter. PFS was defined as the period from the date of initial diagnosis to the date of progression or last follow-up. OS was defined as the length of time from either the date of diagnosis or the start of treat-

ment for EOC in patients alive at the last follow up. *BRCA* testing results were categorized as *BRCA* mutation, wild-type, or VUS.

4. Statistical analysis

Categorical variables were compared between groups using the χ^2 test. The Kaplan-Meier method with log-rank test was used to compare PFS and OS of wild-type vs. mutant groups. Statistical analysis was performed using R software (https://www.r-project.org/) version 3.2. A 2-sided *P*-value less than 0.05 was considered to be statistically significant.

Results

1. Prevalence of *BRCA1/2* mutation

A total of 3,726 patients were treated at Samsung Medical Center between January 1, 2007 and January 1, 2017, and 313 patients (8.4%) who underwent the *BRCA* test were eligible for the current study. Of the 313 patients, 88 (28.1%) had a *BRCA* mutation. Fifty-seven patients (18.2%) had a *BRCA1* mutation, while 31 patients (9.9%) had a *BRCA*2 mutation. In addition, 48 patients (15.3%) were identified with *BRCA1/2* VUS. Among them, 27 patients (8.6 %) had *BRCA1* VUS, while 21 patients (6.7%) had *BRCA2* VUS.

2. Patient characteristics

Patient characteristics were compared according to *BRCA1/2*

Table 1. Patient characteristics by *BRCA1* status

Factors	*BRCA1* WT (n=229)	*BRCA1* VUS (n=27)	*BRCA1* mutation (n=57)	*P*-value
Age (yr)	51.0 (45.0–58.0)	49.0 (42.0–51.0)	49.0 (45.0–57.0)	0.115
Stage				0.489
I, II	67 (30.2)	7 (25.9)	15 (26.8)	
III	117 (52.7)	15 (55.6)	36 (64.3)	
IV	38 (17.1)	5 (18.5)	5 (8.9)	
Histology				0.101
High-grade serous	184 (81.1)	17 (65.5)	53 (93.1)	
Low-grade serous	13 (5.7)	5 (19.2)	2 (3.5)	
Endometrioid	5 (2.2)	1 (3.8)	0 (0.0)	
Mucinous	8 (3.5)	1 (3.8)	1 (1.7)	
Clear cell	13 (5.7)	2 (7.7)	0 (0.0)	
Others	4 (1.8)	0 (0.0)	1 (1.7)	
Grade				0.394
1	14 (6.5)	2 (9.1)	2 (3.5)	
2	37 (17.3)	4 (18.2)	16 (28.1)	
3	163 (76.2)	16 (72.7)	39 (68.4)	
Residual disease status after primary cytoreductive surgery				0.141
0–9 mm	168 (73.4)	23 (85.2)	37 (64.9)	
≥1 cm	61 (26.6)	4 (14.8)	20 (35.1)	
Pre-operative serum CA-125 level (U/mL)	502.0 (106.0–1,611.0)	336.0 (68.0–1,005.0)	907.0 (271.0–1,990.0)	0.092
Neoadjuvant chemotherapy				0.924
Yes	22 (9.6)	2 (7.4)	5 (8.8)	
No	207 (90.4)	25 (92.6)	52 (91.2)	

Values are expressed as median (range) or number (%).
WT, wild-type; VUS, variant of unknown significance; CA, cancer antigen.

mutation and VUS, as shown in Tables 1 and 2. The median age at diagnosis was 49 years for *BRCA1* mutation (range, 45–57 years) and 53 years for *BRCA2* mutation (ranging from 48–58.5 years). Most patients showed serous type disease on histology. A larger proportion of patients with high-grade serous disease was observed in the *BRCA1* mutation group (93.1%) than in the *BRCA1* wild-type (81.1%) or *BRCA1* VUS (65.5%) groups, but no significant difference based on *BRCA* status was observed. Similar patterns of histologic distribution were observed in *BRCA2* mutations. The proportion of advanced stages (FIGO stage III/IV) did not differ among *BRCA1* wild-type, *BRCA1* mutation, and *BRCA1* VUS groups (69.8% for *BRCA1* wild-type, 73.2 % for *BRCA1* mutation, and 74.1% for *BRCA1* VUS). In contrast, *BRCA2* VUS patients showed a significantly larger proportion of FIGO stage IV than did the *BRCA2* mutation and *BRCA2* wild-type patients (14.9% in *BRCA2* wild-type vs. 3.3% in *BRCA2* mutation vs. 45% in *BRCA2* VUS, *P*=0.002). Over 90% of the total patients had high-grade (grade 2 or 3) pathology, and no meaningful difference based on *BRCA* status was observed. There was no significant association between *BRCA1* mutation and optimal primary debulking rate (73.4% for *BRCA1* wild-type, 64.9% for *BRCA1* mutation, 85.2% for *BRCA1* VUS), and a similar pattern was observed for *BRCA2* mutations.

CA-125 level before primary debulking surgery was not significantly different according to *BRCA* status.

Twenty-nine (9.2%) patients were treated with neoadju-

Table 2. Patient characteristics by *BRCA2* status

Factors	*BRCA2* WT (n=261)	*BRCA2* VUS (n=21)	*BRCA2* mutation (n=31)	*P*-value
Age (yr)	50.0 (45.0–57.0)	53.0 (48.0–62.0)	53.0(48.0–58.5)	0.209
Stage				0.002
I, II	76 (29.8)	4 (20.0)	9 (30.0)	
III	141 (55.3)	7 (35.0)	20 (66.7)	
IV	38 (14.9)	9 (45.0)	1 (3.3)	
Histology				0.102
High-grade serous	208 (80.6)	15 (71.3)	31 (100)	
Low-grade serous	17 (6.6)	3 (14.3)	0 (0.0)	
Endometrioid	5 (1.9)	1 (4.8)	0 (0.0)	
Mucinous	9 (3.5)	1 (4.8)	0 (0.0)	
Clear cell	14 (5.5)	1 (4.8)	0 (0.0)	
Others	5 (1.9)	0 (0.0)	0 (0.0)	
Grade				0.514
1	16 (6.6)	2 (10.5)	0 (0.0)	
2	48 (19.8)	4 (21.1)	5 (16.1)	
3	179 (73.7)	13 (68.4)	26 (83.9)	
Residual disease status after primary cytoreductive surgery				0.799
0–9 mm	191 (73.2)	14 (66.7)	23 (74.2)	
≥1 cm	70 (26.8)	7 (33.3)	8 (25.8)	
Pre-operative serum CA-125 level (U/mL)	497.5 (110.0–1,858.5)	646.0 (124.0–1,248.5)	641.0 (186.5–1,522.5)	0.825
Neoadjuvant chemotherapy				0.473
Yes	26 (10.0)	2 (9.5)	1 (3.2)	
No	235 (90.0)	19 (90.5)	30 (96.8)	

Values are expressed as median (range) or number (%).
WT, wild-type; VUS, variant of unknown significance; CA, cancer antigen.

vant chemotherapy followed by interval debulking surgery. The response rate of primary platinum-based chemotherapy in our study population was 89.1% (279/313). Among 88 patients who had a *BRCA* mutation, 26 (29.5%) were treated with PARPi.

Brief review of patient's characteristics between *BRCA1* VUS and *BRCA2* VUS were shown in Supplementary Table 1.

3. Survival outcomes

With a median follow-up duration of 19.8 months (ranging from 2–120 months), 193 patients showed disease progression, and 19 patients died due to EOC. Median PFS for *BRCA1* wild-type, *BRCA1* mutation, and *BRCA1* VUS was 18.9, 23.0, and 19.1 months, respectively, and 19.4, 31.2, and 18.2 months for *BRCA2* wild-type, *BRCA2* mutation, and *BRCA2* VUS, respectively. No significant difference was detected in terms of PFS between patients with *BRCA1* mutation and *BRCA1* wild-type or VUS (*P*=0.10, *P*=0.49, respectively) (Fig. 1A-C).

BRCA1 VUS did not show meaningful difference in PFS compared to *BRCA1* mutation (*P*=0.87) (Fig. 1D). Regardless of *BRCA1* mutation, there was no significant difference in OS (Supplementary Fig. 1A). In contrast, the presence of *BRCA2* mutation was associated with longer PFS compared to *BRCA2* wild-type (*P*=0.04) (Fig. 2A and B). The PFS curve of *BRCA2* VUS showed no meaningful difference from that of *BRCA2* wild-type (*P*=0.41) (Fig. 2C). The *BRCA2* mutation group showed significantly higher PFS compared to *BRCA2*

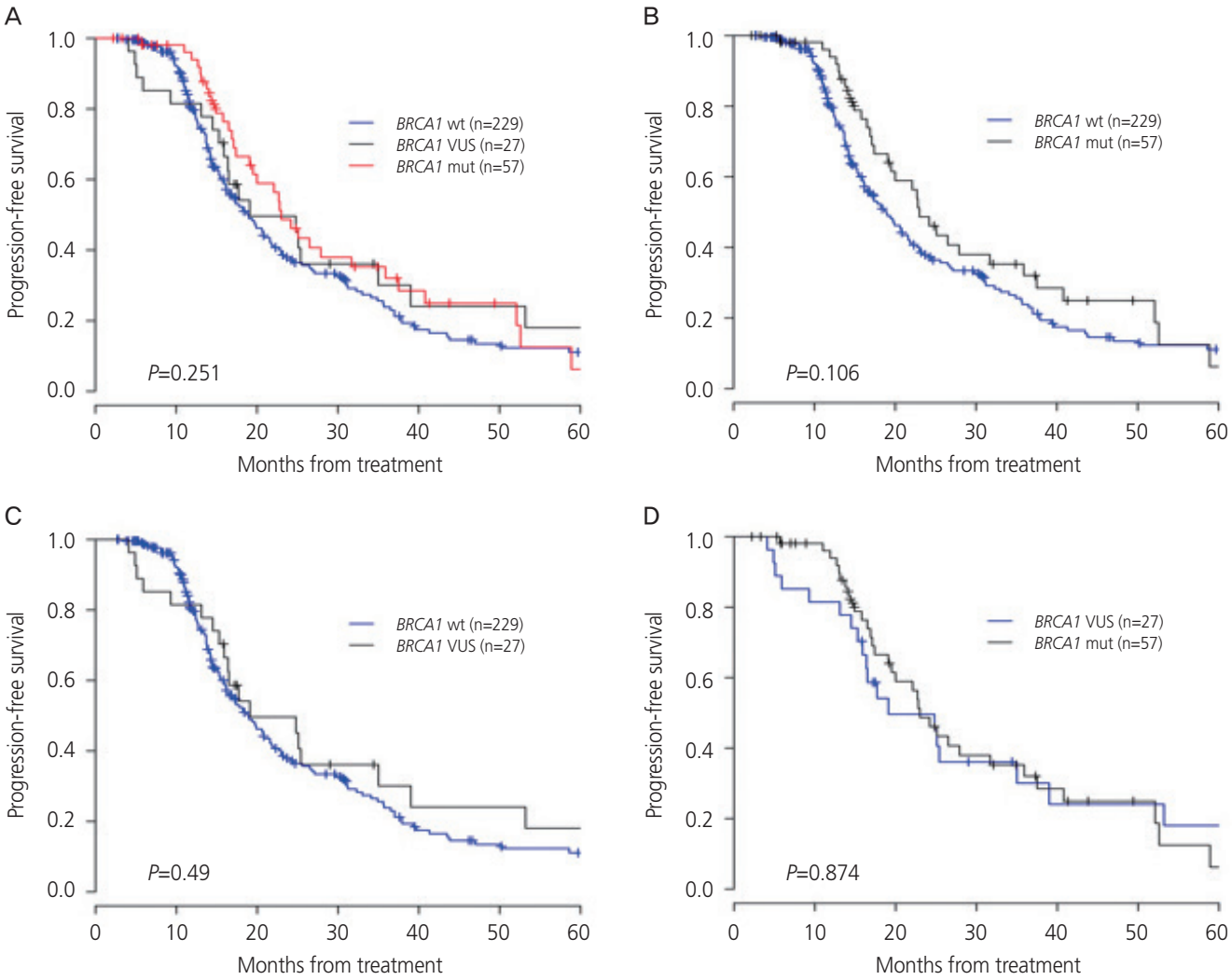

Fig. 1. Kaplan-Meier curves of progression-free survival (PFS) by *BRCA1*. VUS, variant of unknown significance; wt, wild type; mut, mutation.

VUS (*P*=0.02) (Fig. 2D). In contrast to PFS rates, there was no difference in OS between *BRCA2* mutation (including VUS status) and *BRCA2* wild-type (Supplementary Fig. 1B).

Brief review of clinical outcomes between *BRCA1* VUS and *BRCA2* VUS were shown in Supplementary Table 1.

Discussion

A number of previous studies have shown improved clinical outcomes in patients with *BRCA1* or *BRCA2* mutation compared to patients with wild-type *BRCA* in EOC [11,12]. Those patients received platinum-based chemotherapy as a first-line standard therapy for ovarian cancer and showed improved outcomes with *BRCA1* or *BRCA2* mutation related to impairment in homologous recombination-mediated DNA repair. Other studies have shown that patients with *BRCA2* mutation had improved OS and PFS compared to those with *BRCA1* mutation [3,4]. However, such studies excluded *BRCA* VUS results and only focused on patients with *BRCA1/2* mutation. Only a few reports have analyzed VUS prevalence in Asian patients. According to such studies, only about 7% of EOC patients had *BRCA* VUS [13,14]. Until recently, there has been a paucity of evidence regarding the pathogenicity

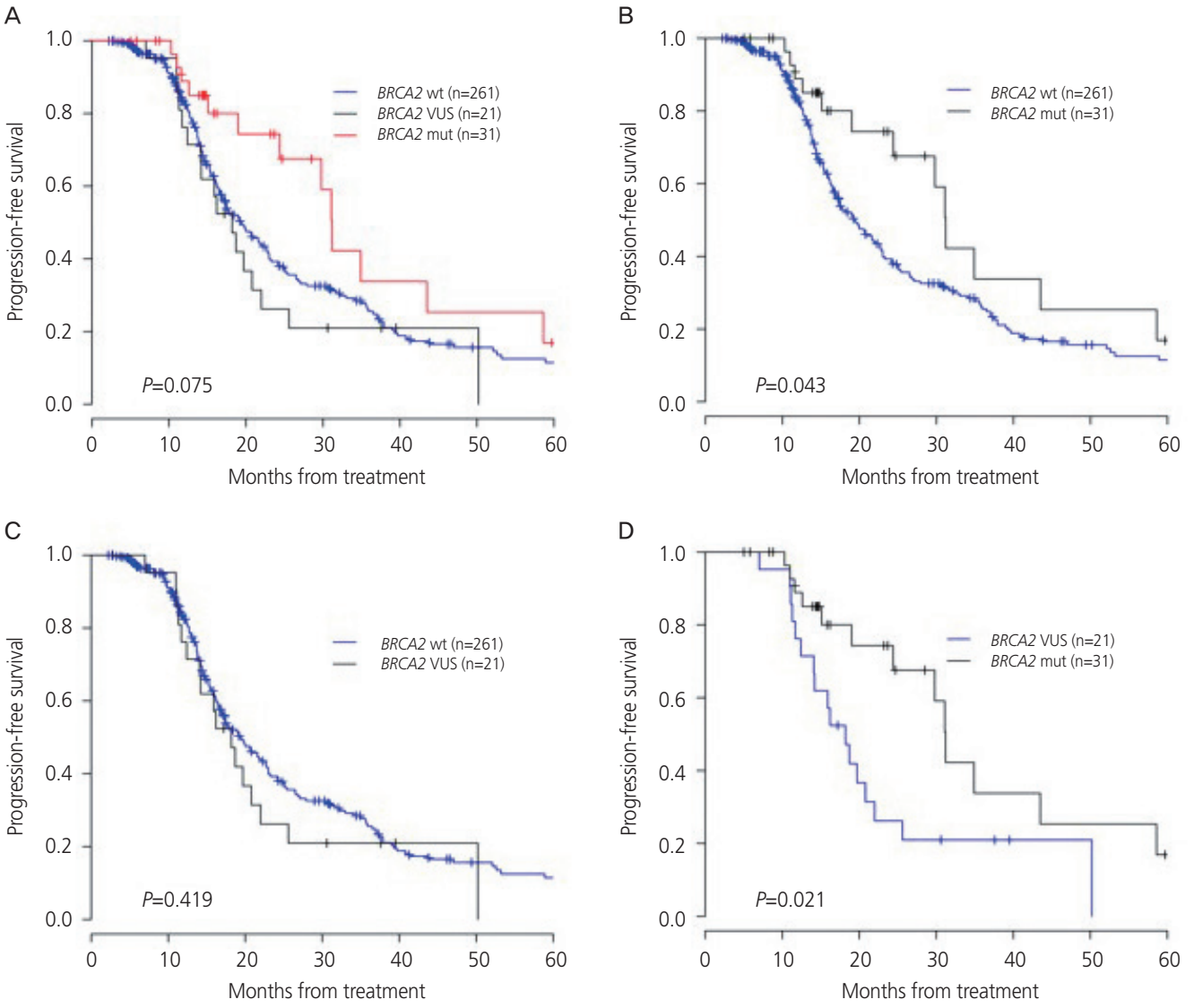

Fig. 2. Kaplan-Meier curves of progression-free survival (PFS) by *BRCA2*. VUS, variant of unknown significance; wt, wild type; mut, mutation.

of *BRCA* VUS in EOC [15]. Some studies revealed that *BRCA* VUS should be treated as *BRCA* wild-type based solely on patient history, but clinical outcomes were not analyzed [16,17]. Regarding *BRCA* mutation, the SOLO-2 study reported that platinum-sensitive, recurrent EOC patients have better PFS when treated with PARPi [18]. However, that study did not include patients with *BRCA* VUS, and further research is needed to determine the benefit of PARPi application in such patients.

This retrospective case-control study investigated baseline characteristics and clinical outcomes of EOC patients with *BRCA1/2* mutation or VUS. The *BRCA* mutation rate was 28% in our study population, which is consistent with previously reported results ranging from 5% to 29% [5,19-26]. The *BRCA1/2* VUS rate was 15% (48/313). Favorable results regarding *BRCA* VUS were identified in this study, in that such patients did not show significant difference in PFS or OS compared to those with *BRCA1* mutation or *BRCA1* wild-type. Patients with *BRCA2* VUS were significantly associated with lower median PFS compared to those with *BRCA2* mutation. The strength of this study is inclusion of a large number of patients who underwent *BRCA* gene mutation testing. The present study is also valuable in that it is the first study comparing clinical outcomes of *BRCA* mutation to *BRCA* VUS according to subtype of EOC histology in the Korean population. This study provides clinical characteristics and survival outcomes of *BRCA1/2* VUS in EOC patients and offers a good starting point for further research.

This study has several limitations. One of the limitations of this study is its retrospective nature. In patients with recurrent EOC, survival outcomes were not separated by chemotherapy duration. Due to a low death rate (19/313, 6.0%), the median OS of our study showed no meaningful difference in *BRCA1/2* mutations or VUS. This is because many of the patients were diagnosed recently and had only a short follow-up time. When follow-up time is prolonged, there might be a significant difference in survival outcomes. In addition, only a small number of patients with *BRCA1/2* mutation or VUS were enrolled, which can limit the significance of this study. Multi-center studies focusing on *BRCA1/2* mutation and VUS are needed and may reveal differences in OS due to *BRCA* mutation or *BRCA* VUS.

In conclusion, our study shows similar patient demographics in *BRCA* mutation, wild-type, and VUS groups. When comparing survival outcomes, *BRCA2* mutation showed improved PFS compared to *BRCA2* wild-type or *BRCA2* VUS. *BRCA1* mutation did not produce significant difference in OS or PFS compared to *BRCA* wild-type.

Conflict of interest

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

Ethical approval

The study was approved by the Institutional Review Board of Samsung Medical Center (IRB No. 2019-05-080-001) and performed in accordance with the principles of the Declaration of Helsinki.

Patient consent

Informed consent was waived because of the retrospective study design.

Supplementary materials

Supplementary materials associated with this article can be found online at https://doi.org/10.5468/ogs.2019.62.6.411.

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