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Distinct Clinical Courses of Epithelial Ovarian Cancer with Mutations in *BRCA1* 5' and 3' Exons

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Abstract. *Background/Aim: This study aimed to determine the effect of different BRCA1 exonal mutations on the clinical course of epithelial ovarian cancer (EOC). Patients and Methods: Clinicopathological variables and survival outcomes were compared among 53 primary EOC patients with pathogenic BRCA1 mutations in exons 1-11 (5' mutations) and in exons 12-24 (3' mutations). Results: BRCA1 5' exonal mutations were found in 35 (66.0%) patients. The median follow-up period was 40 months. Clinicopathological variables remained unchanged between the two groups. Patients with 5' mutations had a significantly longer progression-free survival than those with C-terminal mutations (p=0.034), better predicting progression-free survival [2.923 (1.402-6.093), p=0.004], but not overall survival in cases of multiple relapses (p=0.497). Conclusion: N-terminal BRCA1 mutations in EOC patients are associated with favourable primary progression-free survival, a trend observed only in primary progression-free survival, not in overall survival.*

Epithelial ovarian cancer (EOC) remains a gynaecologic malignancy with the highest mortality, with over two-thirds of patients diagnosed at advanced stages of the disease (1). Approximately 10% of cases are due to an inherited predisposition, and a large fraction of the inheritance is attributable to mutations in *BRCA1/2* (2). Several previous studies have reported that germline mutations in *BRCA1* and *BRCA2* are correlated with a favourable prognosis, probably owing to the high response rate of their carriers to platinum-based chemotherapy (3-7).

However, conclusive evidence on *BRCA1*-associated prognosis in EOC patients is lacking because of inconsistent data on the survival advantage of mutated carriers compared to non-mutated patients, particularly regarding long-term follow-up (8, 9). Moreover, the results of these studies do not consider the location of the mutated exon within *BRCA1*.

BRCA1 contains 24 exons encoding a large protein of 1,863 amino acid residues (10). However, limited information is available regarding the clinical effects of mutations located in different *BRCA1* exons on EOC onset and progression (11). The available evidence allows the assumption that mutations in different *BRCA1* gene sites may differentially affect the clinical course of ovarian cancer disease (12, 13). Therefore, in the present study, the clinical impact of *BRCA1* mutations located in the first 11 exons was compared with that of mutations located in the last 13 exons, with a focus on clinicopathological characteristics and survival outcomes.

Materials and Methods

Patient selection and pathologic review. The study protocol was approved by the Institutional Review Board of the Yonsei University College of Medicine (No. 4-2017-0564), and the study was performed in accordance with the ethical standards described in the Declaration of Helsinki. Informed consent was waived by the Institutional Review Board of the Yonsei University College of Medicine because of the retrospective nature of the study that based on medical records, and the fact that this research presented no more than minimal risk of

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Key Words: BRCA1, epithelial ovarian cancer, exon, prognosis.

Table I. *Clinicopathological characteristics of patients.*

Variables	Total (n=53)	Mutated exon level		
		Exon number ≤11 (n=35)	Exon number >11 (n=18)	*p*-Value
Age at diagnosis, year	49 (27-77)	49 (28-77)	52.5 (27-72)	0.11
Family history	23 (43.4)	14 (40.0)	9 (50.0)	0.242
Breast cancer history	21 (39.6)	12 (34.3)	9 (50.0)	
Histology				
Serous	52 (98.1)	35 (100.0)	17 (94.4)	0.159
Endometrioid	1 (1.9)	0	1 (5.6)	
Stage				
Early (1, 2)	4 (7.5)	2 (5.7)	2 (11.1)	0.481
Advanced (3, 4)	49 (92.5)	33 (94.3)	16 (88.9)	
Grade				
Low	1 (1.9)	0	1 (5.6)	0.222
High	52 (98.1)	35 (100.0)	17 (94.4)	
Peritoneal carcinomatosis	29 (54.7)	22 (62.9)	7 (38.9)	0.097
CA125 ≥500 U/ml	32 (60.4)	22 (62.9)	12 (66.7)	
NAC	12 (22.6)	6 (17.6)	6 (33.3)	0.3
Residual disease				
NGR	26 (49.1)	15 (42.9)	11 (61.1)	0.537
<0.5 cm	17 (32.1)	12 (34.3)	5 (27.8)	
<1.0 cm	7 (13.2)	6 (17.1)	1 (5.6)	
<2.0 cm	3 (5.7)	2 (5.7)	1 (5.6)	

NAC: Neoadjuvant chemotherapy; NGR: no gross residual disease.

harm to subjects. EOC patients with *BRCA1* mutations were included in the analysis. Upon reviewing their medical records, data regarding several characteristics of the patients, including age at diagnosis, histological type (from pathology reports), and surgical stage in accordance with the International Federation of Gynecology and Obstetrics (FIGO) criteria were obtained. A homogeneous regimen (*i.e.* paclitaxel and carboplatin) was administered as postoperative adjuvant chemotherapy six times in cases involving primary debulking surgery and of postoperative adjuvant chemotherapy thrice followed by six times in cases involving neoadjuvant chemotherapy. The patients were divided into two groups on the basis of the location of the mutated exon in *BRCA1*. The mutation was defined as 5' *BRCA1* mutation if it occurred in exons 1-11, and as a 3' *BRCA1* mutation if it occurred in exons 12-24. Clinicopathological variables and survival outcomes in patients with mutations in exons 1-11 (5' mutations) were compared to those in patients with mutations in exons 12-24 (5'nal mutations). Exon 11 was considered to represent the central region of *BRCA1*. Platinum sensitivity was defined as the maintenance of complete remission for 12 months or more after the completion of frontline chemotherapy.

Direct sequencing. Genetic testing for *BRCA1* (accession number NM_007294) mutations was performed *via* direct sequencing, as described previously (14). Genetic mutations analysed herein were confined to deleterious mutations, such as frameshift or nonsense mutations. Variations were described in accordance with the nomenclature system of the Human Genome Variation Society (http://www.hgvs.org/mutnomen) and the conventional nomenclature system from the Breast Cancer Information Core (BIC; http://research.nhgri.nih.gov/bic/).

Statistical analysis. Statistical analysis was performed using IBM SPSS version 23.0 for Windows (SPSS Inc., Chicago, IL, USA). Pearson's chi-squared test, Fisher's exact test, and Mann–Whitney *U*-test were performed for univariate analysis. Survival outcomes were determined *via* Kaplan–Meier survival analysis. First progression-free survival (PFS) was defined as the interval between the date of initiation of treatment and the date of documented disease progression or death from any cause. Second and third PFSs were defined as the intervals between the date of termination of prior treatment and the date of documented disease progression or death from any cause. Overall survival (OS) was defined as the interval between the date of initiation of treatment and the date of death due to any cause. Patients lost to follow-up were censored at the last date of contact. Univariate and multivariate analyses of the effects of various prognostic factors on survival were performed using the Cox proportional hazards model. Multivariate analysis was carried out using variables with *p*-Value less than 0.1 in the univariate analysis.

Results

Patient characteristics. Patient characteristics are enlisted in Table I. In total, 53 EOC patients with *BRCA1* mutations were included. The distribution of mutations in exons is presented in Figure 1. Mutations in *BRCA1* exons 1-11 were present in 35 of the 53 patients (66.0%), whereas mutations in *BRCA1* exons 12-24 were observed in 18 patients (34.0%). There were no significant differences in clinicopathological variables, including age at diagnosis, family history of cancer, breast

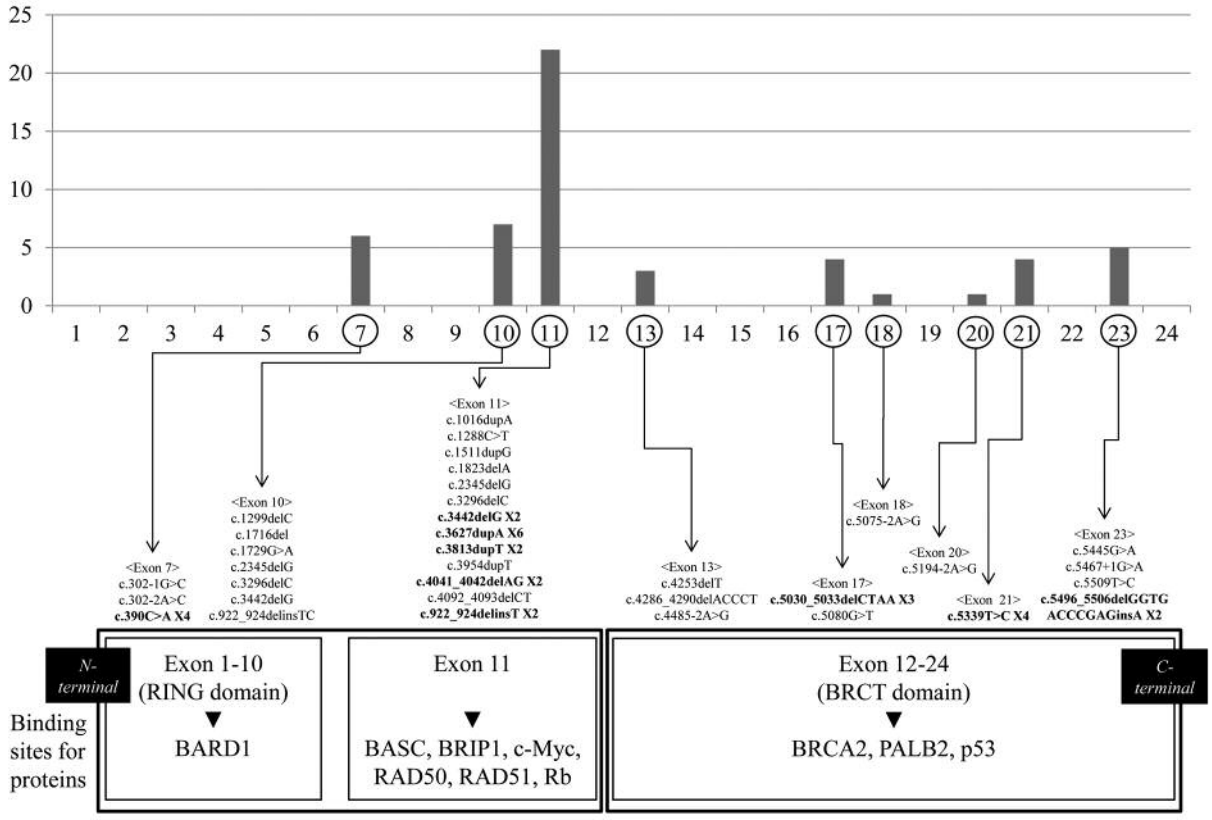

Figure 1. *Distribution of mutations in BRCA1 exons in ovarian cancer patients and reported binding sites for proteins (20). Data in boldface indicate repeated detection.*

cancer history, histology, grade, FIGO stage, peritoneal carcinomatosis, CA125 higher than 500, the decision of whether to administer neoadjuvant chemotherapy, or residual disease after debulking surgery in patients with N-terminal mutations and those with C-terminal *BRCA1* mutations.

Sensitivity to chemotherapy. The proportion of patients displaying chemosensitivity is presented in Figure 2. The presence of a germline mutation in *BRCA1* exons 1-11 was closely associated with primary chemotherapy sensitivity, with a relative risk (RR) of 1.43 [95% confidence interval (CI)=1.082-2.079]. However, the ability of genetic alterations to predict responses to chemotherapy was lost in the second and third rounds of chemotherapy, with RR of 0.947 (95%CI=0.385-2.332) and RR of 1.061 (95%CI=0.363-3.101), respectively.

Survival analysis. The median follow-up period was 40 months. Kaplan–Meier survival analysis revealed significantly improved PFS in patients with 5' *BRCA1* mutations in exons 1-11 compared with that in patients with 3' *BRCA1* mutation in exons 12-24 (p=0.034, Figure 3A). However, the tendency of favourable outcomes was not observed in patients experiencing multiple relapses (Figure 3B and C). In particular, no significant difference in OS was observed between the two groups of patients (p=0.497, Figure 3D). The presence of a 5' *BRCA1* mutation was found to be one of the significant predictive factors for PFS upon Cox regression multivariate analysis [2.923 (1.402-6.093), p=0.004] and upon univariate analysis [3.865 (1.583-9.439), p=0.003], as was the residual disease after debulking surgery [2.267 (1.065-4.829), p=0.034] (Table II).

Discussion

The present study aimed to assess the clinical impact of mutations in the *BRCA1* gene depending on the mutation site. The results indicate that EOC patients with mutations at the 5' and 3' parts of the *BRCA1* gene (exons 1-11 and 12-24, respectively) did not differ with respect to their clinicopathologic variables, including optimal debulking rate and FIGO stage. However, the first chemotherapy response rate and first PFS were significantly higher in patients with 5' *BRCA1* mutations. This trend was not observed after

Figure 2. *Proportion of patients displaying chemosensitivity.*

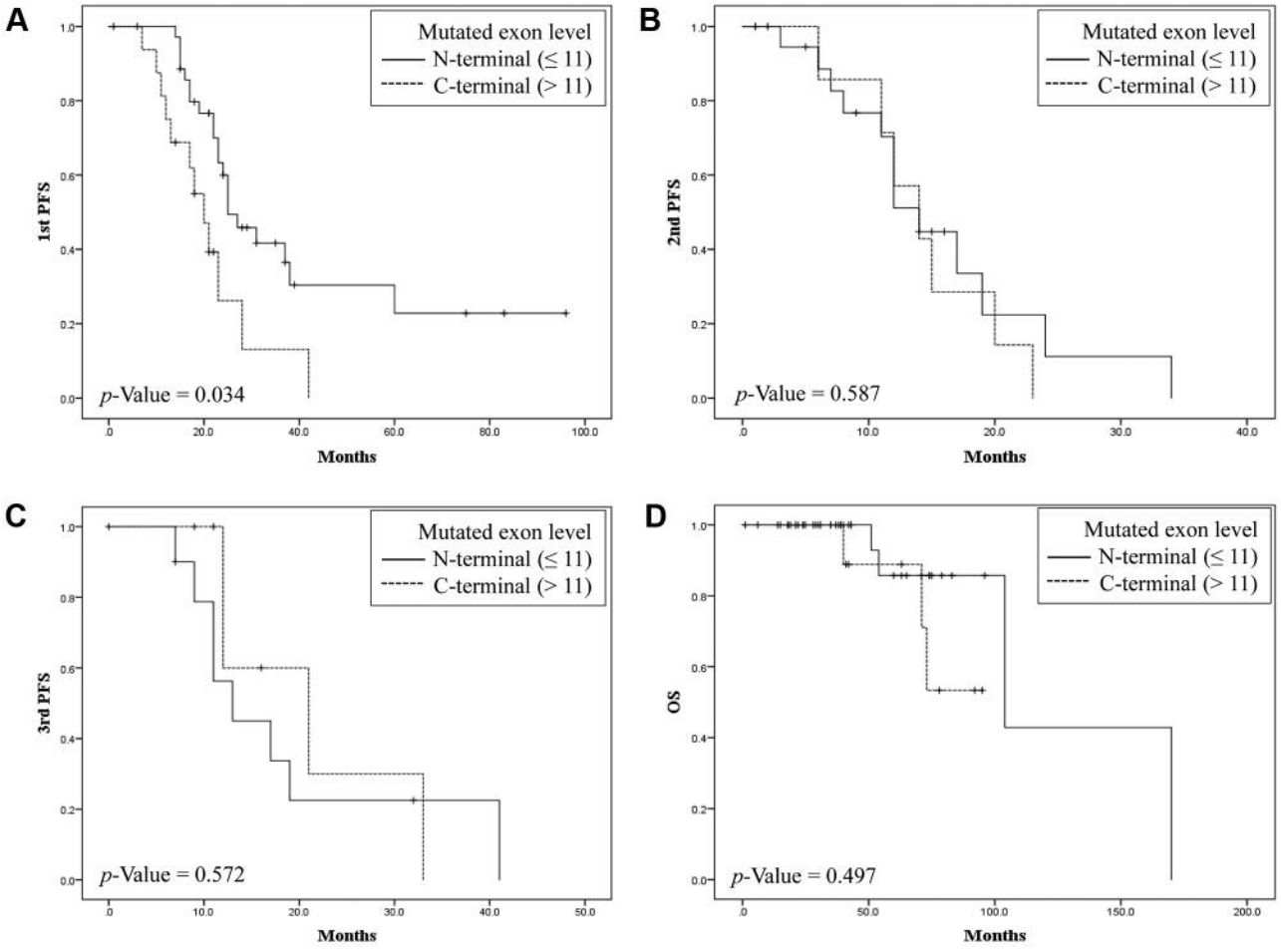

Figure 3. *Comparison of the 1st PFS (A), 2nd PFS (B), 3rd PFS (C), and OS (D) in groups of patients with 5' and 3' BRCA1 exon mutations. PFS: Progression-free survival; OS: overall survival.*

Table II. *Univariate and multivariate analyses of prognostic factors for progression-free survival.*

	No. of patients	PFS			
		Univariate analysis		Multivariate analysis	
		HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age, years (continuous)	53	0.993 (0.955-1.032)	0.724		
Stage					
Advanced	49	1 (Reference)			
Early	4	0.527 (0.098-2.844)	0.327		
Preoperative CA 125, U/ml					
≤500	21	1 (Reference)			
>500	32	1.488 (0.666-3.327)	0.333		
Peritoneal carcinomatosis					
No	24	1 (Reference)			
Yes	29	1.542 (0.624-3.813)	0.348		
Breast cancer history					
No	32	1 (Reference)			
Yes	21	0.940 (0.421-2.101)	0.88		
Cancer family history					
No	30	1 (Reference)			
Yes	23	0.858 (0.386-1.910)	0.708		
NAC					
No	41	1 (Reference)	0.454		
Yes	12	0.489 (0.091-2.636)			
Residual disease					
NGR	26	1 (Reference)		1 (Reference)	
Non-NGR	27	2.243 (0.963-5.224)	0.061	2.267 (1.065-4.829)	0.034
Mutated exon level					
≤11 (N-terminal)	35	1 (Reference)		1 (Reference)	
>1 (C-terminal)	18	3.865 (1.583-9.439)	0.003	2.923 (1.402-6.093)	0.004

PFS: Progression-free survival; HR: hazard ratio; CI: confidential interval; NAC: neoadjuvant chemotherapy; NGR: no gross residual disease.

multiple relapses and in the OS. With accurate validation, these data may have implications for decision making related to risk assessment and cancer prevention during the treatment of patients harbouring *BRCA1* mutations.

Although the clinical role of *BRCA1* germline mutations has been investigated in detail in EOC patients, comprehensive data regarding the association between the location of *BRCA1* mutations and the natural course of EOC are still wanted. We hypothesized that mutations in different *BRCA1* exons might differentially affect the response to double-strand break (DSB)-inducing agents. Our results suggest that mutations in 5' exons might result in a lower residual function of the BRCA1 protein compared with those in 3' exons.

The effect of different *BRCA1* gene mutation sites on patient prognosis has been reported previously (13). However, contrary to our results, the previous study reported that 5' BRCA1 mutations correlated with decreased survival, whereas 3' mutations were associated with increased survival. Concurrent with those findings, another recent study reported that mutations in the RING-type zinc finger domain (5') of *BRCA1* confer poor responsiveness to cisplatin and poly ADP ribose polymerase inhibitors (12). In the present study, we observed that 5' mutations in exons 1-11 of the *BRCA1* gene result in decreased residual activity of the BRCA1 protein, seemingly correlating with an increased response rate to cytotoxic chemotherapy. However, this trend was not observed after multiple recurrences. This discrepancy warrants further analysis in studies with larger samples.

DSBs are the most cytotoxic forms of DNA damage (15). In addition, homologous recombination (HR) deficiency is known to underlie the hypersensitivity of *BRCA1*-deficient cells to DNA DSB-inducing agents (16). Although patients with *BRCA1*-associated EOC are often sensitive to platinum-based chemotherapy, they eventually develop chemoresistance through secondary mutations in *BRCA1*, which restore its function (17). It is unclear which functions of *BRCA1* confer chemotherapeutic resistance; however, the reversion of *BRCA1* germline mutations has been observed in resistant tumors (18).

BRCA1 is a large protein that comprises four domains required for heterodimerization of BRCA1 and numerous HR-related enzymes, including BARD1, BRIP1, the RAD family, PALB2, and BRCA2 (Figure 1) (19). Our

observations are concurrent with the concept that once a mutation in *BRCA1* has occurred, residual gene function of *BRCA1* might be a critical factor for the repair process. Mutations in different exons may produce differential effects on the clinical course of EOC by altering interactions of BRCA1 with various types of HR-related enzymes.

One of the most remarkable findings of the present study is that the difference in the fraction of chemosensitive patients and in the tendency of favourable primary PFS was obliterated in patients experiencing multiple relapses. The trend of favourable outcomes of *BRCA1*-associated EOC patients is reportedly lost in long-term follow-up periods (9, 20). This might be explained by the reversal of *BRCA1* mutations observed in recurrent cancer tissue, which is assumed to be a resistance mechanism (21). According to our data, we assumed that 5' mutations in exons 1-11 of *BRCA1* appeared prone to reversal in multiple relapses, thereby resulting in chemoresistance. This hypothesis warrants further validation with sequential biopsy for the analysis of somatic mutations in future studies.

One limitation of the present study is that the assessments were performed in a small cohort in a retrospective manner. Furthermore, clinical effects of all types of mutations in *BRCA1* were assessed. However, to our knowledge, this is the first study based on the concept that exon location may influence the clinical course of *BRCA1*-associated EOC. Moreover, EOC patients were treated similarly at a single institution, and the prevention of a potential bias, including the exclusion of patients with *BRCA2* mutations, can be highlighted as a strength of the present study.

In summary, 5' mutations in *BRCA1* in exons 1-11 apparently confer a more favourable primary PFS in EOC patients with a higher response rate to chemotherapy. However, this trend of favourable prognosis was observed only in primary PFS. For personalized medicine, future studies on *BRCA1* gene mutations should focus on the clinical features of different types of mutations, particularly their location on different exons. This process might enable selection of patients with *BRCA1* mutations benefitting maximally from treatment with new targeted therapies, including those involving poly ADP ribose polymerase inhibitors. Such studies will also provide access to new drugs in a more cost-effective manner.

Conflicts of Interest

The Authors have no financial conflicts of interest to disclose regarding this study.

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