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ORIGINAL REPORT

# Somatic Mutations in *BRCA1* and *BRCA2* Could Expand the Number of Patients That Benefit From Poly (ADP Ribose) Polymerase Inhibitors in Ovarian Cancer

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## ABSTRACT

### Purpose
The prevalence of *BRCA1/2* mutations in germline DNA from unselected ovarian cancer patients is 11% to 15.3%. It is important to determine the frequency of somatic *BRCA1/2* changes, given the sensitivity of *BRCA*-mutated cancers to poly (ADP ribose) polymerase-1 (PARP1) inhibitors and platinum analogs.

### Patients and Methods
In 235 unselected ovarian cancers, *BRCA1/2* was sequenced in 235, assessed by copy number analysis in 95, and tiling arrays in 65. 113 tumors were sequenced for *TP53*. *BRCA1/2* transcript levels were assessed by quantitative polymerase chain reaction in 220. When available for tumors with *BRCA1/2* mutations, germline DNA was sequenced.

### Results
Forty-four mutations (19%) in *BRCA1* (n = 31)/*BRCA2* (n = 13) were detected, including one homozygous *BRCA1* intragenic deletion. *BRCA1/2* mutations were particularly common (23%) in high-grade serous cancers. In 28 patients with available germline DNA, nine (42.9%) of 21 and two (28.6%) of seven *BRCA1* and *BRCA2* mutations were demonstrated to be somatic, respectively. Five mutations not previously identified in germline DNA were more commonly somatic than germline (four of 11 *v* one of 17; $P = .062$). There was a positive association between *BRCA1* and *TP53* mutations ($P = .012$). *BRCA1/2* mutations were associated with improved progression-free survival (PFS) after platinum-based chemotherapy in univariate ($P = .032$; hazard ratio [HR] = 0.65; 95% CI, 0.43 to 0.98) and multivariate ($P = .019$) analyses. *BRCA*1/2 deficiency, defined as *BRCA1/2* mutations or expression loss (in 24 [13.3%] *BRCA1/2*–wild-type cancers), was present in 67 ovarian cancers (30%) and was also significantly associated with PFS in univariate ($P = .026$; HR = 0.67; 95% CI, 0.47 to 0.96) and multivariate ($P = .008$) analyses.

### Conclusion
*BRCA1/2* somatic and germline mutations and expression loss are sufficiently common in ovarian cancer to warrant assessment for prediction of benefit in clinical trials of PARP1 inhibitors.

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## INTRODUCTION

*BRCA1* and *BRCA2* play a critical role in DNA repair by homologous recombination.[1] *BRCA1/2* germline mutations occur in 11% to 15.3% of women with unselected ovarian cancers.[2-4] Poly (ADP-ribose) polymerase-1 (PARP1) inhibitors are synthetic lethal with *BRCA1/2* dysfunction in homologous recombination–deficient cancers and are currently in clinical trials in *BRCA1/2* germline mutation carriers with ovarian and breast cancer.[5] The preliminary results of these studies are encouraging.[6] Because PARP1 inhibitors may also be effective in cancers in which *BRCA1/2* and thus homologous recombination function is compromised by somatic aberrations, the number of women with ovarian cancer who might benefit from PARP1 inhibitors may be greater than predicted by the frequency of germline *BRCA1/2* mutations. However, *BRCA1/2* status has not been comprehensively studied in a large cohort of unselected human ovarian cancers to assess whether loss of *BRCA* function can also occur due to somatic events. We thus evaluated *BRCA1/2* in 235 unselected ovarian cancers by sequencing, identifying intragenic deletions, determining gene copy number, and quantifying expression of *BRCA1/2*

 

using the assays described below. Germline mutation status was determined in patients whose tumors demonstrated *BRCA1/2* aberrations when normal DNA could be obtained.

## PATIENTS AND METHODS

### Patient Characteristics

Human ovarian cancer tissues (n = 235) were obtained from the Gynecology Cancer Banks at The University of Texas M. D. Anderson Cancer Center and University of California San Francisco under institutional review board–approved protocols (Table 1). The cases were randomly selected from all ovarian cancers with available snap-frozen tumor tissue collected between June 1990 and December 2006. As varying numbers of samples were used in the assays below, Table 2 provides the rationale for why only a subset of cancers were assessed in specific assays.

### RNA/DNA Extraction From Frozen Cancers

Sections of 10-$\mu$m thickness from frozen cancers in Tissue-Tek OCT (Qiagen, Valencia, CA) were homogenized using a TissueRuptor (Qiagen) after adding QIAzol lysis reagent, followed by RNA isolation using a QIAgen miRNAeasy MiniKit per manufacturers protocol. A QIAamp DNA MiniKit (Qiagen) was used to isolate DNA per manufacturer's protocol with overnight incubation (56°C) and RNaseA treatment.

### Quantitative Polymerase Chain Reaction

Reverse transcription was performed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) per manufacturer instructions. For preamplification, a 0.2× probe mix was made by combining 1 $\mu$L of 91 20X gene expression assays from Applied Biosystems

**Table 1.** Patient and Cancer Characteristics

| Characteristic | No. of Patients | % | Mutations in Each Group | | | *P* for *BRCA1/2* Mutations by Each Clinical Variable |
|---|---|---|---|---|---|---|
| | | | Total | *BRCA1* | *BRCA2* | |
| Total patients | 235 | | | | | |
| Age at diagnosis, years | | | | | | |
| Range | 23-92 | | <60:27* | 21 | 7 | .013 |
| Median | 60 | | >60:13 | 9 | 4 | |
| Unknown | 20 | 8.5 | 3 | 1 | 2 | |
| Follow-up time, days | | | | | | |
| Range | 19-6,241 | | NA | | | NA |
| Median | 1,071 | | | | | |
| Unknown | 8 | 3.5 | | | | |
| Stage | | | | | | |
| 1 | 11 | 5 | 2 | 2 | 0 | .121 |
| 2 | 14 | 6 | 1 | 1 | 0 | |
| 3 | 156 | 66 | 27* | 19 | 9 | |
| 4 | 33 | 14 | 10 | 8 | 2 | |
| Unknown | 21 | 9 | 3 | 1 | 2 | |
| Histology | | | | | | |
| Serous | 186 | 79 | 39* | 30 | 10 | .102 |
| Nonserous | 13 | 6 | 0 | 0 | 0 | |
| Mixed | 13 | 6 | 1 | 0 | 1 | |
| Unknown | 22 | 9 | 3 | 1 | 2 | |
| Grade | | | | | | |
| 1 | 13 | 5.5 | 0 | 0 | 0 | .044 |
| 2 | 19 | 8 | 3 | 2 | 1 | |
| 3 | 180 | 76.5 | 37* | 28 | 10 | |
| Unknown | 23 | 10 | 3 | 1 | 2 | |
| Residual disease after surgery, cm | | | | | | |
| 0 | 12 | 5 | 2 | 2 | 0 | .945 |
| ≤ 1 | 126 | 53.5 | 25 | 18 | 7 | |
| > 1 | 60 | 25.5 | 11* | 8 | 4 | |
| Unknown | 37 | 16 | 5 | 3 | 2 | |
| Surgery | | | | | | |
| Yes | 230 | 98 | NA | | | NA |
| No | 5 | 2 | | | | |
| Unknown | 0 | | | | | |
| Chemotherapy | | | | | | |
| No chemotherapy | 9 | 3.8 | NA | | | NA |
| Unknown | 33 | 14 | | | | |
| Platinum (cisplatin or carboplatin) based (no taxane) | 17 | 7.2 | | | | |
| Platinum plus taxane (paclitaxel or docetaxel) based | 176 | 74.9 | | | | |

Abbreviation: NA, not applicable.
*The patient with a tumor harboring both a *BRCA1* and BRCA2 mutation is included in the groups indicated and was counted only once in each analysis. Patients with missing clinical data were not included in the analysis.

**Table 2.** Number of Ovarian Cancers Used in Each Assay

| Assay | No. of Samples | If the Assay Was Not Applied to All 235 Ovarian Cancer Cases, the Reason for This Was as Follows |
|---|---|---|
| Sequencing: *BRCA1/2* | 235 | — |
| Quantitative PCR of *BRCA1* and *BRCA2* expression | 220 | 220 samples with RNA of sufficient quality were used for quantitative PCR |
| Affymetrix 500K SNP arrays | 95 | Samples with sufficient DNA remaining after completion of other DNA assays listed were used; the ultra high-density SNP array data were of sufficient quality for assessment of *BRCA1/2* gene copy numbers in 95 cases |
| Sequencing-*TP53* | 113 | Samples with sufficient high-quality DNA were selected randomly to determine whether an association existed between *TP53* and *BRCA1/2* mutations |
| High-density tiling array: *BRCA1/2* | 65 | Samples were selected randomly. The assay was discontinued after 65 cases due to low yield (only one of 65 intragenic deletions [in *BRCA1*] was detected) |

Abbreviations: PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism.

and 9 $\mu$L of low-EDTA Tris-EDTA (TE). Preamplification was performed using 2.5 $\mu$L of 2× TaqMan PreAmp Master Mix (Applied Biosystems), 1.25 $\mu$L 0.2× probe mix, and 1.25 $\mu$L of cDNA. Applied Biosystems TaqMan assays (*BRCA1*: Hs00173233_m1/Hs00173237_m1/Hs01556190_m1/Hs01556191_m1; *BRCA2*: Hs00609060_m1; housekeepers: Hs99999908_m1 [*GUSB*]/Hs00188166_m1 [*SDHA*]/Hs00237047_m1 [*YWHAZ*]/Hs00824723_m1 [*UBC*]/Hs00609297_m1 [*HMBS*]) were used for preamplification and quantitative polymerase chain reaction (PCR) on a Fluidigm (South San Francisco, CA) BioMark instrument. Cycle conditions were 95°C × 10 minutes, 17 cycles of 95°C × 15 seconds, and 60°C × 4 minutes. The PCR products were diluted 1:5 with low-EDTA TE. Samples were assessed on gene expression M48 dynamic arrays (Fluidigm) per manufacturer's protocol. The comparative cycle threshold (Ct) method was used to calculate relative gene expression using the Ct for the *BRCA2* assay, the average Cts from the *BRCA1* assays, and the average Cts from housekeeper genes. Quantitative PCR was performed in 220 cancers for which high-quality RNA was obtained.

### Mutation Screening

PCR was performed on 2 ng of DNA in a 3-$\mu$L reaction using the primers flanking the exons of *BRCA1/2* that are used in the BRCAnalysis (Myriad Genetics, Salt Lake City, UT) clinical test with the following cycling conditions: 95°C × 10 minutes, 35 cycles of 95°C × 30 seconds, 62°C × 30 seconds, and 72°C × 1 minute, finishing with 72°C × 1 minute. Each PCR product was treated with 0.1 U of Shrimp Alkaline Phosphatase (Sigma-Aldrich, St Louis, MO.) The PCR product was diluted 1:9, and 0.8 $\mu$L was used for cycle sequencing with Big Dye Sequencing Chemistry and Taq FS (Applied Biosystems). Cycle conditions were 95°C × 3 minutes, 32 cycles of 95°C × 30 seconds, 50°C × 30 seconds, 60°C × 3 minutes, and 72°C × 10 minutes. Sequence products were run on a Megabace 4500 automated sequencer (GE Medical Systems, Milwaukee, WI) per manufacturer's protocol.

*BRCA1/2* mutations were only included in the analyses below if classified as deleterious or suspected deleterious based on established criteria.[7] A suspected deleterious mutation typically is treated clinically as deleterious.

*TP53* was amplified in 113 cancers with sufficient DNA using nested PCR. Primary PCR was performed using Taq-Platinum and 1 $\mu$L of 2 ng/$\mu$L of DNA in a 3-$\mu$L reaction with primers without M13 tails. The PCR product was diluted nine-fold and used for a secondary reaction with primers that have M13 tails. Sequence products were run on a Megabace 4500 automated sequencer per the manufacturer's protocol.

### High-Density Tiling Array

The array was designed using eArray (Agilent Technologies, Santa Clara, CA) and synthesized on a 8 × 15,000 probe format. The array design included probes spaced at 20–base-pair intervals across the complete genomic region of *BRCA1/2* from 10 kb upstream of the 5′UTR to 5 kb downstream of the 3′UTR avoiding repeats. Additional probes (1,000) were evenly distributed across the genome to form a backbone against which specific genomic gain/loss was estimated.

Sample preparation/array processing was performed using the Oligonucleotide Array-Based CGH for Genomic DNA Analysis kit and protocol (Agilent Technologies). These arrays were run on 65 ovarian cancers. Data were analyzed using DNA Analytics 4.0 (version 4.0.76) software (Agilent Technologies).

### Affymetrix 500K Single-Nucleotide Polymorphism Arrays

In 203 cancers for which sufficient DNA was present, 250 ng of genomic DNA was processed using GeneChip Mapping NspI or StyI Assay Kit (Affymetrix, Santa Clara, CA) per manufacturer's protocol and hybridized to Affymetrix Mapping 500K NspI or StyI microarrays. After hybridization, array wash, stain, and scan procedures were performed per manufacturer's protocol. Copy number/loss of heterozygosity (LOH) analysis were performed using a software package described elsewhere (Abkevich V et al, manuscript in preparation). Ninety-five chips with high-quality data were used for the final analysis.

### Statistical Analysis

All analyses were carried out using R, v2.9.0 (www.*R*-project.org). In all analyses, observations were removed if the response or a covariate was missing. Fisher's exact test was used to make comparisons involving pairs of categorical variables. *t* test was used to compare differences in means of continuous variables. Cox proportional hazards regression was used to perform univariate and multivariate analysis on the progression-free survival (PFS) and overall survival (OS) times from the date of debulking surgery. Comparisons of survival probabilities for categorical variables were visualized with Kaplan-Meier plots. The partial likelihood ratio test was used to compute *P* values. Wald statistic-based CIs were calculated for hazard ratio (HR) point estimates.

## RESULTS

### BRCA1/2 Mutations in Ovarian Cancers

In DNA extracted from 235 human ovarian cancers (Table 1), 44 mutations in *BRCA1* (n = 31) and *BRCA2* (n = 13) were detected in 43 tumors, including one small homozygous intragenic *BRCA1* deletion that was detected using tiling arrays. In one cancer, both a *BRCA1* and *BRCA2* mutation was detected. All but one of the mutations included in the analyses are known or predicted deleterious mutations, with only one mutation being a suspected deleterious mutation (*BRCA1*, *G1738R*).[7] Five novel mutations were not present in the Myriad Genetics or BIC[8] germline *BRCA1/2* mutation databases (Appendix Table A1, online only). This *BRCA1/2* mutation frequency of 19% observed in tumor tissue is higher than the expected frequency of germline mutations in an unselected population of patients with ovarian cancer (11% to 15.3%).[2-4] In 212 tumors with known grade, no *BRCA1/2* mutations were observed in grade 1 cancers (0 of 13 *v* 40 of

  

199; $P = .135$; see Table 1 for a detailed list of missing data for each clinical variable). No mutations were found in patients with tumors without a serous component as compared with those with tumors of pure serous histology (0 of 13 *v* 39 of 186; $P = .076$). When stage was known, *BRCA1/2* mutation status was not significantly associated with stage (stage 1 or 2 disease [three of 25] *v* stage 3 or 4 disease [37 of 189]; $P = .584$). The frequency of *BRCA1/2* mutations in high-grade (grade 3) serous cancers was 22.8% (36 of 158).

### *Germline Versus Somatic* BRCA1/2 *Mutations in Ovarian Cancers*

Of the 43 patients with ovarian cancers harboring a *BRCA1* or *BRCA2* mutation (note that one patient had an ovarian cancer with two mutations, that is, in *BRCA1* and *BRCA2*), germline DNA was available from 28 patients. In these 28 patients, 11 (39.3%; 95% CI, 22.1 to 59.3) ovarian tumor *BRCA1* (nine of 21; 42.9%) and *BRCA2* (two of seven; 28.6%) mutations could be demonstrated to be somatic due to an inability to detect the aberration in germline DNA, whereas 17 mutations (60.7%) were found in both tumor and germline DNA. No significant differences were found between germline and somatic *BRCA1/2*-mutant cancers in terms of any clinical variables, although the low tumor numbers limited the power of these analyses. Interestingly, somatic mutations were more frequently novel mutations (four of 11), as defined by absence from the Myriad Genetics or BIC[8] germline *BRCA1/2* mutation databases, than germline mutations (one of 17; $P = .062$). No somatic mutations were detected in tumors from patients with germline mutations.

### *Homozygous* BRCA1/2 *Deletions in Ovarian Cancers*

One homozygous intragenic deletion in *BRCA1* and none in *BRCA2* was detected by high-density tiling arrays in 65 ovarian cancers. Homozygous deletion of both copies of *BRCA1* or *BRCA2* was not detected by 500K single-nucleotide polymorphism array in 95 patients with high-quality single-nucleotide polymorphism data, confirming the low frequency of deletions in tumors.

### *LOH of* BRCA1/2 *in Ovarian Cancers*

LOH in *BRCA1* was detected in 82 (87.2%) of 94 ovarian cancers. In contrast, LOH in *BRCA2* was detected in significantly fewer (45 [52.9%] of 85; $P < .0001$) ovarian cancers. The one retained gene copy was replicated (two or three copies) in 28 of 45 cases of LOH of *BRCA2* (62.2%) and in 51 of 82 cases of LOH at *BRCA1* (62.2%). Interestingly, LOH of *BRCA2* was only detected in one sample without LOH of *BRCA1* ($P = .001$).

### *Association of* BRCA1/2 *Aberrations With Expression Levels*

Neither the expression of *BRCA1* ($P = .684$, n = 220) nor *BRCA2* ($P = .966$, n = 220) was significantly different in *BRCA1*- or *BRCA2*-mutated cancers as compared with wild-type cancers, respectively (Figs 1A and 1B). In other words, *BRCA* expression level was not influenced significantly by *BRCA* mutation status. Similarly, *BRCA1* and *BRCA2* LOH was not associated with significantly reduced expression of *BRCA1* or *BRCA2*, respectively, possibly because of duplication of the retained copy of the gene in a significant proportion of cases of LOH (as described above).

We also hypothesized that loss of expression of *BRCA1* or *BRCA2* in ovarian cancer would, as with *BRCA1* or *BRCA2* mutations, impair the function of *BRCA1* or *BRCA2*. Cancers without *BRCA1/2* mutations were considered to have loss of *BRCA* expression if the average of *BRCA1* and *BRCA2* $\delta C_T$ (threshold for expression level of *BRCA1/2* corresponding to the 95th percentile of *BRCA1/2* expression in *BRCA1/2* mutant tumors) was higher than the 95th percentile of a normal distribution fit to the mutants' average $\delta C_T$. As defined in this way, loss of *BRCA* expression was present in 24 (13.3%) *BRCA1/2*–wild-type cancers, implicating other potential mechanisms (eg, methylation) in loss of *BRCA1* and *BRCA2* gene expression.

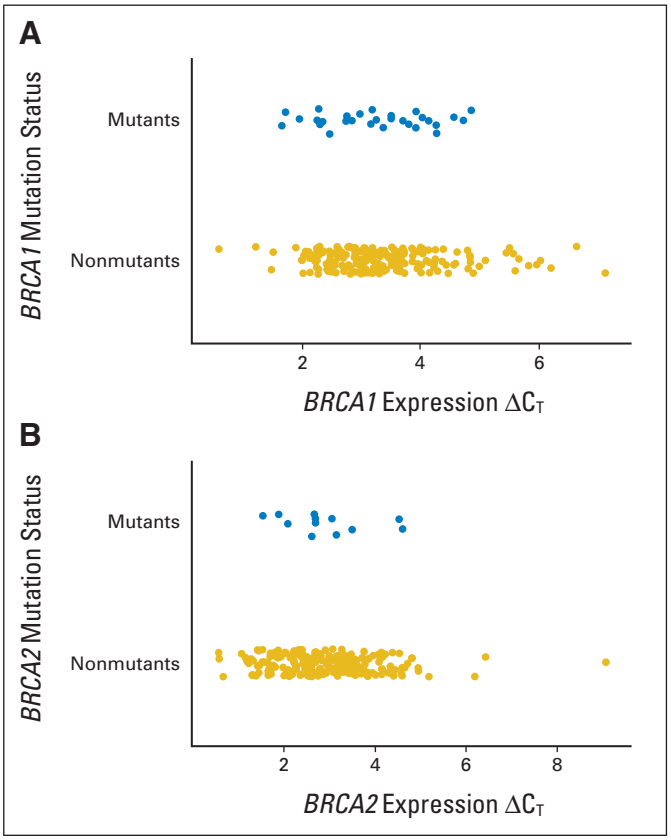


**Fig 1.** The relationship between (A) *BRCA1* mutations and *BRCA1* gene expression and between (B) *BRCA2* mutations and *BRCA2* gene expression is shown. The average $\delta C_T$ of *BRCA1* for *BRCA1* mutants (3.26, n = 29) is not significantly different from the average $\delta C_T$ of *BRCA1* for *BRCA1* nonmutants (3.33, n = 191; *P* value = 0.68). Likewise, the averages of $\delta C_T$ of *BRCA2* for *BRCA2* mutants (2.92, n = 12) and nonmutants (2.90, n = 208) are not significantly different ($P = .97$).

### *Survival Associations*

Germline mutations in *BRCA1* and *BRCA2* have been reported to be associated with improved outcomes for patients with ovarian cancer after surgery and platinum-based chemotherapy.[9-11] Likewise, herein, *BRCA1* and *BRCA2* mutations in ovarian cancer tissue were associated with a significantly improved PFS as compared with *BRCA1/BRCA2*–wild-type cancers in univariate analysis (Fig 2; $P = .032$; HR = 0.65; 95% CI, 0.44 to 0.98). This significant association was maintained in a multivariable Cox model that included clinical variables (Table 3). PFS for individuals with germline *BRCA* mutations was not significantly different from that of individuals with somatic *BRCA* mutations ($P = .690$), albeit with low numbers. In contrast, *BRCA1* and *BRCA2* mutations together in ovarian cancer

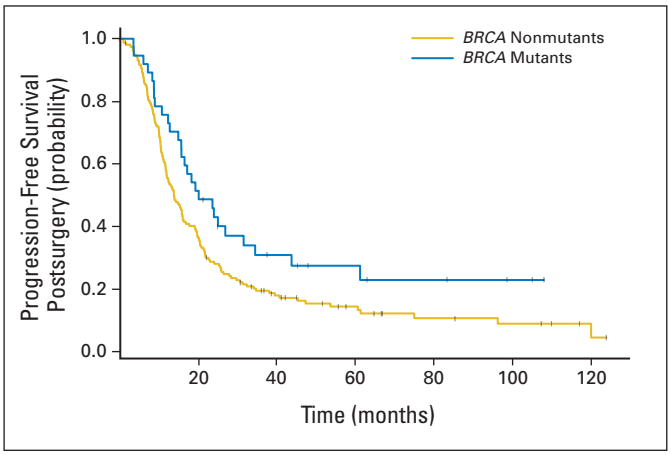


**Fig 2.** Kaplan-Meier curves showing that *BRCA1* and *BRCA2* mutations together in ovarian cancer tissue were associated with significantly improved progression-free survival (PFS) time after surgery as compared with *BRCA1*– and *BRCA2*–wild-type ovarian cancers. Median PFS for *BRCA* mutants and nonmutants were 20.1 (95% CI, 15.6 to 43.8) and 13.8 (95% CI, 11.9 to 16.3) months, respectively ($P = .032$). The patient with both a *BRCA1* and a *BRCA2* mutation was only counted once.

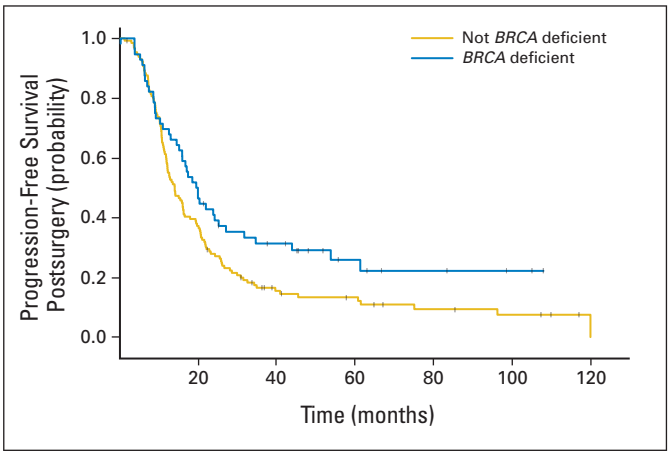


**Fig 3.** Kaplan-Meier curves showing that *BRCA1/2* deficiency in ovarian cancer tissue was associated with significantly improved progression-free survival (PFS) time after surgery as compared with other ovarian cancers. Median PFS for cancers with and without *BRCA* deficiency were 19.3 (95% CI, 15.7 to 31.6) and 13.8 (95% CI, 11.9 to 16.3) months, respectively ($P = .026$). The patient with both a *BRCA1* and a *BRCA2* mutation was only counted once.

tissue were not significantly associated with OS. An integrated dichotomous *BRCA1/2*-deficiency variable was defined as the presence of mutations or loss of expression of *BRCA1* or *BRCA2*. This *BRCA1/2* deficiency variable was present in 67 (30.0%) of 223 ovarian cancers. In univariate analyses, *BRCA1/2* deficiency was significantly associated with PFS (Fig 3; $P = .026$; HR = 0.67; 95% CI, 0.47 to 0.96). This significant association of *BRCA1/2* deficiency with PFS was maintained in a multivariable Cox model (Table 4).

Neither LOH of *BRCA1* nor of *BRCA2* was significantly associated with PFS or OS times compared with other ovarian cancers.

### *Associations Between* BRCA1 *and* BRCA2 *and Other Mutations*

*TP53* mutations were present in 81 (71.7%) of 113 cases and were significantly associated with *BRCA1* and marginally associated with all *BRCA1/2* mutations (Appendix Table A2, online only). Given the association of mutations in *BRCA1* and of both *BRCA1/2* mutations with *TP53* mutations, we hypothesized that if LOH represented loss of *BRCA* function, it should also be associated with *TP53* mutations. Indeed, we found that *TP53* mutations were significantly associated with LOH in *BRCA1* (54 of 63 *v* 0 of 10; $P < .0001$) and marginally associated with LOH in *BRCA2* (28 of 34 *v* 18 of 30; $P = .057$).

## DISCUSSION

This is the first comprehensive study of *BRCA1/2* status in ovarian cancer tissue. Although thought previously to be uncommon, we demonstrate that somatic *BRCA1/2* mutations account for at least one third of *BRCA1/2* mutations in ovarian cancer specimens.[12] In fact, *BRCA1/2* mutations in total occur in approximately 18% of all ovarian cancers and 23% of high-grade serous cancers, compared with previous reports that *BRCA1/2* germline mutations occur in 11% to 15.3% of unselected women with ovarian cancer.[2-4] Using cases for which germline DNA was available, we estimate germline and somatic mutation rates of approximately 11.5% and 7%, respectively. Mutations of *BRCA1/2* in ovarian cancers are associated with improved PFS after surgery and platinum/taxane-based chemotherapy. This is consistent with several previous reports for germline *BRCA1* and *BRCA2* mutations in women with ovarian cancer and likely represents, at least in part, increased effectiveness of platinum drugs in cancer cells with deficient homologous recombination.[1,7,9,10] We also hypothesized that loss of expression of *BRCA*1 or *BRCA2* in ovarian cancer would, as with *BRCA1/BRCA2* mutations, impair *BRCA1/2* function and

**Table 3.** Multivariable Cox Model of Progression-Free Survival in Women With Ovarian Cancer

| Variable | *P* | Hazard Ratio | 95% CI |
|---|---|---|---|
| Residual disease | .003 | 1.80 | 1.24 to 2.59 |
| Stage | .002 | 2.43 | 1.30 to 4.54 |
| Grade | .027 | 1.76 | 1.03 to 2.99 |
| *BRCA1/2* mutation status | .019 | 0.61 | 0.39 to 0.94 |

NOTE. Residual disease was a binary variable (0 for ≤ 1 cm and 1 for > 1 cm). Stage was a binary variable (0 for stages 1 and 2 and 1 for stages 3 and 4). Grade was a binary variable (0 for grades 1 and 2 and 1 for grade 3). The patient with a tumor harboring both a *BRCA1* and a *BRCA2* mutation was only counted once.

**Table 4.** Multivariable Cox Models of Progression-Free Survival in Women With Ovarian Cancer

| Variable | *P* | Hazard Ratio | 95% CI |
|---|---|---|---|
| Residual disease | .002 | 1.86 | 1.27 to 2.72 |
| Stage | .002 | 2.40 | 1.28 to 4.48 |
| Grade | .029 | 1.74 | 1.02 to 2.96 |
| *BRCA1/2* deficiency | .008 | 0.60 | 0.41 to 0.89 |

NOTE. Residual disease was a binary variable (0 for ≤ 1 cm and 1 for > 1 cm). Stage was a binary variable (0 for stages 1 and 2 and 1 for stages 3 and 4). Grade was a binary variable (0 for grades 1 and 2 and 1 for grade 3). The patient with a tumor harboring both a *BRCA1* and a *BRCA2* mutation was only counted once.

  

thus significantly improve PFS after surgery and platinum-based chemotherapy. Indeed, *BRCA1/2* deficiency (mutations plus expression loss) was also associated with PFS, suggesting that loss of *BRCA1* and *BRCA2* expression likely occurs for reasons other than mutations and rare homozygous deletions and may also impair homologous recombination in cancer cells. Of note, the numbers of low *BRCA1/2* expressors in our study were not consistent with reported rates of methylation of these genes in our studies or in the literature (approximately 20%), perhaps because methylation may not in all cases lead to the very low level of *BRCA* expression defined in our article.[13] However, it is possible that published studies have overestimated the methylation rate or that our study may underestimate the rate of low *BRCA* expression in ovarian cancer.

In contrast, and although some studies suggest an OS benefit in patients with germline *BRCA1/2* mutations,[14] mutations of *BRCA1/2* in ovarian cancers were not significantly associated with OS herein. This may reflect, at least in part, a lack of impact of *BRCA1/2* mutations on ovarian cancer responsiveness to nonplatinum chemotherapy drugs used as second-line therapy or on the natural history of ovarian cancer in the absence of therapy. After all, platinum sensitivity, but not sensitivity to other cytotoxic chemotherapy drugs commonly used in ovarian cancer treatment, is a surrogate marker for impaired homologous recombination in cancer cells. Alternatively, we may have seen an OS benefit with *BRCA1/2* mutations with larger patient numbers. Although neither PFS nor OS was significantly different in individuals with germline versus somatic *BRCA1/2* mutations, our current data set is not large enough to enable us to definitively determine whether there is a biologic difference between germline and somatic mutations.

Mutations of *BRCA1* are almost universally associated with *TP53* mutations. This is consistent with genetically engineered mouse models in which *BRCA1* deletion is an early lethal, whereas embryos with combined *BRCA1* and *TP53* mutations survive significantly longer.[15]

Several reasons may explain the lack of an association between *BRCA* transcript expression levels and mutations or LOH. In the case of *BRCA1/2* LOH, in approximately 62% of cases, there were two (and, in the case of *BRCA1,* sometimes three) copies of the gene. In the case of mutations, a significant proportion of the mutations identified do not result in nonsense mediated transcript decay. We also identified LOH in many tumors without mutations (87% and 53% of tumors had LOH at *BRCA1* and *BRCA2* but only 13% and 6% had *BRCA1* and *BRCA2* mutations, respectively). As in all ovarian tumors with *BRCA1/2* LOH, in tumors with *BRCA1* or *BRCA2* LOH but without mutations, we observed approximately 60% of cases with two or more gene copies and almost 40% with only one copy. Thus if loss of one gene copy does affect gene expression, then this would affect both *BRCA1/2*-mutant and nonmutant ovarian tumors. These and likely other factors may explain the lack of a correlation between *BRCA1/2* expression and either *BRCA1/2* mutation status or LOH.

Two studies have proposed that platinum chemoresistance can arise from mutations that restore the *BRCA2* open reading frame and thus homologous recombination.[16,17] In this study, there was no evidence of somatic mutations in ovarian cancers from patients with germline mutations. Because the cancer tissues used in this study were mostly collected before chemotherapy administration, our study does not impinge on the concept that selective pressure during chemotherapy could select for mutations that restore *BRCA* function.

Currently, germline *BRCA1/2* mutation screening is performed in women with ovarian cancer judged to be at high risk for carrying an inherited mutation based on clinical models (eg, BRCAPRO).[18,19] PARP1 inhibitor trials are underway in *BRCA1/2* germline mutation carriers with ovarian cancer, and the preliminary results of these studies are encouraging.[5,6] However, because PARP1 inhibitors are selectively active in *BRCA1/2*-deficient cancers, assessment of *BRCA1/2* mutation status in all ovarian cancers could identify a higher number of women who might benefit from these novel drugs. This approach should be investigated in future trials of PARP1 inhibitors. Whether loss of expression or LOH of *BRCA1* or *BRCA2* will contribute to sensitivity to PARP1 inhibitors also warrants exploration. Further, homologous recombination is a complex process with multiple components. Thus the frequency of homologous recombination aberrations will be greater than that indicated by studies of *BRCA1/2* alone. Although that frequency is difficult to speculate at this time, it is likely that additional patients with ovarian cancer may benefit from PARP inhibitors.

In summary, loss of *BRCA* function due to frequent somatic aberrations in ovarian cancers likely deregulates homologous recombination and thereby increases sensitivity to platinum drugs and possibly also to PARP1 inhibitors. This is consistent with prior studies of germline *BRCA1/2* mutations. The novel observation that somatic *BRCA1/2* aberrations occur frequently could significantly increase the ability to identify patients who will benefit from PARP1 inhibitors in ovarian cancer clinical trials. Somatic and germline mutations and *BRCA1/2* expression loss are sufficiently common in ovarian cancer to warrant assessment in clinical trials for prediction of benefit from PARP1 inhibitors.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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