Association of *BRCA1* and *BRCA2* Mutations With Survival, Chemotherapy Sensitivity, and Gene Mutator Phenotype in Patients With Ovarian Cancer

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Context Attempts to determine the clinical significance of *BRCA1/2* mutations in ovarian cancer have produced conflicting results.

Objective To determine the relationships between *BRCA1/2* deficiency (ie, mutation and promoter hypermethylation) and overall survival (OS), progression-free survival (PFS), chemotherapy response, and whole-exome mutation rate in ovarian cancer.

Design, Setting, and Patients Observational study of multidimensional genomics and clinical data on 316 high-grade serous ovarian cancer cases that were made public between 2009 and 2010 via The Cancer Genome Atlas project.

Main Outcome Measures OS and PFS rates (primary outcomes) and chemotherapy response (secondary outcome).

Results *BRCA2* mutations (29 cases) were associated with significantly better OS (adjusted hazard ratio [HR], 0.33; 95% CI, 0.16-0.69; $P=.003$ and 5-year OS, 61% for *BRCA2*-mutated vs 25% for *BRCA* wild-type cases) and PFS (adjusted HR, 0.40; 95% CI, 0.22-0.74; $P=.004$ and 3-year PFS, 44% for *BRCA2*-mutated vs 16% for *BRCA* wild-type cases), whereas neither *BRCA1* mutations (37 cases) nor *BRCA1* methylation (33 cases) was associated with prognosis. Moreover, *BRCA2* mutations were associated with a significantly higher primary chemotherapy sensitivity rate (100% for *BRCA2*-mutated vs 82% [$P=.02$] and 80% [$P=.05$] for *BRCA* wild-type and *BRCA1*-mutated cases, respectively) and longer platinum-free duration (median platinum-free duration, 18.0 months for *BRCA2*-mutated vs 11.7 [$P=.02$] and 12.5 [$P=.04$] months for *BRCA* wild-type and *BRCA1*-mutated cases, respectively). *BRCA2*-mutated, but not *BRCA1*-mutated cases, exhibited a "mutator phenotype" by containing significantly more mutations than *BRCA* wild-type cases across the whole exome (median mutation number per sample, 84 for *BRCA2*-mutated vs 52 for *BRCA* wild-type cases, false discovery rate <0.1).

Conclusion Among women with high-grade serous ovarian cancer, *BRCA2* mutation, but not *BRCA1* deficiency, was associated with improved survival, improved chemotherapy response, and genome instability compared with *BRCA* wild-type.

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INCREASED SURVEILLANCE OF *BRCA1/2* germ line mutation carriers is a generally accepted strategy for detecting early ovarian cancer. Women with *BRCA1* mutations have a 39% to 54% cumulative lifetime risk of developing ovarian cancer and women with *BRCA2* mutations have an 11% to 23% risk.[1-3]

Both *BRCA1* (NCBI Entrez Gene 672) and *BRCA2* (NCBI Entrez Gene 675) tumor suppressor genes are involved in DNA repair via homologous recombination. Cells with alterations in homologous recombination pathway genes are unable to repair DNA double-strand breaks by homologous recombination, which is mostly error free. This can result in genomic instability and a predisposition to malignant transformation.[4,5] Conversely, because homologous recombination pathway deficiencies can also impair tumor cells' ability to repair DNA cross-links introduced by chemotherapy agents such as cisplatin, it has been hypothesized that *BRCA*-deficient patients will likely have higher survival rates because of an improved response to platinum-based chemotherapy.[6]

For editorial comment see p 1597.

However, conflicting data exist regarding the outcome of *BRCA*-deficient patients after ovarian cancer develops. Some researchers have found that ovarian cancer patients with *BRCA1/2* germ line mutations have a more favorable

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clinical course,[7-13] whereas others have shown the opposite.[14,15] Second, whether the effect of *BRCA1/2* mutations on patient outcome is directly attributable to their influence on platinum-based chemotherapy response has not been well elucidated. Most studies that have investigated the clinical features of *BRCA1/2* mutation carriers lack detailed chemotherapy information,[7-10,12,13] apart from occasional studies reporting improved responses to platinum-based therapy in small cohorts.[6]

Using multidimensional genomic and clinical data on 316 high-grade serous ovarian cancer patients in The Cancer Genome Atlas (TCGA) project, we evaluated the association between *BRCA1/2* deficiencies in ovarian cancer and patient overall survival (OS) and progression-free survival (PFS) rates, chemotherapy response, and whole-exome mutation rates.

METHODS

Patients

We searched the TCGA database of 316 high-grade serous ovarian cancer patients on September 1, 2010. Detailed patient information, including age at diagnosis, tumor stage and grade, and surgical outcome, is listed in TABLE 1. All ovarian cancer specimens were surgically resected before systemic treatment and were selected to have greater than 70% tumor cell nuclei and less than 20% necrosis. Ninety-six percent of tumors were stage III or IV, and all were high grade. According to the TCGA database, 86% of patients were non-Ashkenazi Jewish whites, 7% were Ashkenazi Jewish, 3% were African American, and 3% were Asian (Table 1). All patients received a platinum agent and 94% received a taxane.

This analysis was conducted in the Genome Data Analysis Center (GDAC) at The University of Texas MD Anderson Cancer Center and the Institute for Systems Biology. The access to the TCGA database is approved by the National Cancer Institute. The University of Texas MD Anderson Cancer Center waived the requirement for ethical approval of this analysis because the registry is a deidentified database. Written consent was obtained from all live patients.

Duration for OS was defined as the interval from the date of initial surgical resection to the date of death or last contact (censored). The PFS duration was defined as the interval from the date of initial surgical resection to the date of progression/recurrence or last contact (censored). The drug (platinum)-free interval was defined as the interval from the end of adjuvant platinum-based treatment to the date of progression/recurrence or last contact (censored).

Table 1. Age and Tumor Characteristics of Patients With Different *BRCA1/2* Status[a]

Characteristic	All Cases	*BRCA* Wild-Type[b]	*BRCA1* Mutation	*BRCA2* Mutation	*BRCA1* Methylation	*P* Value[c]
No. of cases[d]	316	219	35	27	33	
Age, mean (SD) [range], y	60.6 (0.7) [27-88]	61.8 (0.8) [35-88]	55.9 (1.9) [41-76]	60.9 (2.4) [27-79]	57.3 (1.6) [40-77]	.01
Racial/ethnic background						
Non-Ashkenazi Jewish white	266 (86)	190 (89)	24 (71)	21 (78)	29 (91)	.02
Ashkenazi Jewish	20 (7)	9 (4)	7 (21)	3 (11)	1 (3)	
African American	10 (3)	7 (3)	1 (3)	2 (7)	0	
Asian	10 (3)	6 (3)	2 (6)	0	2 (6)	
Other	2 (1)	1 (1)	0	1 (4)	0	
Missing, No.	8	6	1	0	1	
Tumor stage						
II	14 (4)	9 (4)	2 (6)	1 (4)	2 (6)	.65
III	248 (79)	169 (77)	27 (77)	24 (92)	26 (79)	
IV	53 (17)	41 (19)	6 (17)	1 (4)	5 (15)	
Missing, No.	1	0	0	1	0	
Tumor grade						
2	28 (9)	20 (9)	2 (6)	2 (8)	3 (9)	.92
3	281 (91)	193 (91)	32 (94)	24 (92)	30 (91)	
Missing, No.	7	6	0	1	0	
Residual tumor size, cm						
0[e]	58 (21)	37 (19)	7 (23)	5 (21)	8 (27)	.95
<1	150 (54)	103 (54)	15 (50)	14 (58)	17 (57)	
1-2	14 (5)	11 (6)	2 (7)	1 (4)	0	
>2	56 (20)	41 (21)	6 (20)	4 (17)	5 (17)	
Missing, No.	38	27	5	3	3	

[a] Values are reported as No. (%) unless otherwise indicated. Missing values are excluded from the test calculations.
[b] *BRCA* wild-type cases do not include the *BRCA1* methylation cases.
[c] For categorical data (racial/ethnic background, tumor stage and grade, and residual tumor size), the χ^2 test was used to calculate *P* values; for age as a continuous variable, the Kruskal-Wallis test was used.
[d] Numbers do not sum because 2 cases with *BRCA1* and *BRCA2* mutations were excluded.
[e] Residual tumor size is labeled as 0 cm in patients with no macroscopic disease.

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Analysis of Chemotherapy Response Data

Two aspects of chemotherapy response were investigated: primary response to platinum treatment and platinum-free duration after treatment. The patient was designated as primary sensitive if she had experienced a complete or partial response to adjuvant chemotherapy as noted in TCGA data[16] and as primary resistant if she had stable or progressive disease. On the basis of these criteria, 225 cases were primary sensitive and 36 were primary resistant. Fifty-five cases with no information on primary response to adjuvant therapy were excluded (eFigure 1 available at http://www.jama.com). In 225 primary-sensitive cases, we used the drug (platinum)-free duration to scale chemotherapy response; the shorter the platinum-free duration, the more resistance the patient had. Given that suboptimal debulking can contribute to rapid disease progression,[17] 33 patients with a residual tumor greater than 2 cm were excluded from the platinum-free duration survival analysis (eFigure 1).

Analysis of Whole-Exome Mutation Data

In total, 19 359 mutations across 316 ovarian cancer samples were downloaded from the TCGA Data Portal.[18] The sequencing and quality control procedures were recently described.[16] In brief, whole-exome capture (approximately 180 000 exons from approximately 18 500 genes) and sequencing were performed on 316 ovarian cancer samples and matched (normal) controls. Among them, 236 sample pairs were performed on the Illumina GAIIx platform (Illumina Inc, San Diego, California) and 80 sample pairs on the ABI SOLiD 3 platform (Life Technologies Corp, Carlsbad, California).

We used an enrichment score[19] to determine whether cases with *BRCA1* or *BRCA2* mutations were enriched among hypermutated cases with high mutation rates across the whole exome. First, all 316 ovarian cancer cases were decreasingly ordered on the basis of their total mutation numbers. For each patient group (ie, *BRCA1*- or *BRCA2*-mutated), we calculated the enrichment score, which is a normalized Kolmogorov-Smirnov statistic. Considering the samples $S_1,..,S_i,.., S_{N=316}$, which are ordered on the basis of total mutations, and a patient group P that contains *G* members, we defined

$$X_i = -\sqrt{\frac{G}{N-G}} \quad (1)$$

if S_i was not a member of P and

$$X_i = \sqrt{\frac{N-G}{G}} \quad (2)$$

if S_i was a member of P.

We then computed a running sum across all *N* samples. The enrichment score was defined as

$$ES = \max_{1<j<V} \sum_{i=1}^{j} X_t \quad (3).$$

Intuitively, the enrichment score was calculated by going down the decreasingly ordered sample list. If a sample was included in the target group (ie, *BRCA1*- or *BRCA2*-mutated), we increased the running sum statistic and otherwise decreased the statistic. The enrichment score reached a higher positive score when samples in the target group were consistently ranked at the top of the sample list. The maximum enrichment score was obtained when the *N* samples in the target group were ranked the top *N* most-mutated samples among all 316 ovarian cancer cases. The enrichment score was measured for each *BRCA1*- and *BRCA2*-mutated patient group. To determine whether any given patient group was significantly associated with hypermutation, we permuted the *BRCA* mutation status 10^6 times, which generated a background enrichment score distribution to calculate the false discovery rate.

Analysis of Methylation and Expression Data

Level 3 Illumina Infinium DNA methylation and Agilent 244K gene expression data (Agilent Technologies, Inc, Santa Clara, California) of 316 TCGA ovarian samples and 8 normal fallopian tube samples were downloaded on September 1, 2010, from the Open-Access tiers of TCGA Data Portal.[18] The Illumina Infinium HumanMethylation27 arrays interrogate 27 578 CpG sites located in proximity to the transcription start sites of 14 475 consensus-coding sequences in the NCBI Database (Genome Build 36).

We calculated the Spearman rank correlation between DNA methylation and gene expression for 9 different probes located in 3 CpG islands near the *BRCA1* region and found statistically significant inverse correlations (Benjamini-Hochberg adjusted false discovery rate, <0.0001) for 4 probes (cg19531713, cg19088651, cg08993267, and cg04658354) located in the CpG island near the transcription start site. We then used *K*-means consensus clustering ($K=2$) on the DNA methylation (β values) of 4 probes across 316 samples to separate the epigenetically silenced and nonepigenetically silenced groups of samples. For the 5 probes located in the other 2 CpG islands distant from the transcription start site, no significant inverse correlation with *BRCA1* expression was observed. No inverse correlation between probes near the *BRCA2* region and *BRCA2* mRNA expression was observed.

Statistical Analysis

Standard statistical tests were used to analyze the clinical and genomics data, including the χ^2 test, Fisher exact test, Kruskal-Wallis test, Wilcoxon rank sum test, log-rank test, and Cox proportional hazard analysis. Significance was defined as a *P* value of less than .05. Benjamini-Hochberg multiple testing correction[20] was used to estimate the false discovery rate, when multiple testing correction applied. Analyses were primarily performed using R 2.10.0 (R Foundation for Statistical Computing [http://www.r-project.org/]) and SPSS version 18 (SPSS Inc, Chicago, Illinois).

RESULTS

BRCA1 and *BRCA2* Mutations in Ovarian Cancer

BRCA1 and *BRCA2* were nonsynonymously mutated in 37 (11.7%) and 29 (9.2%) of 316 cases, respectively. Two cases had both *BRCA1* and *BRCA2* mutations and were excluded from analyses comparing *BRCA1*- and *BRCA2*-

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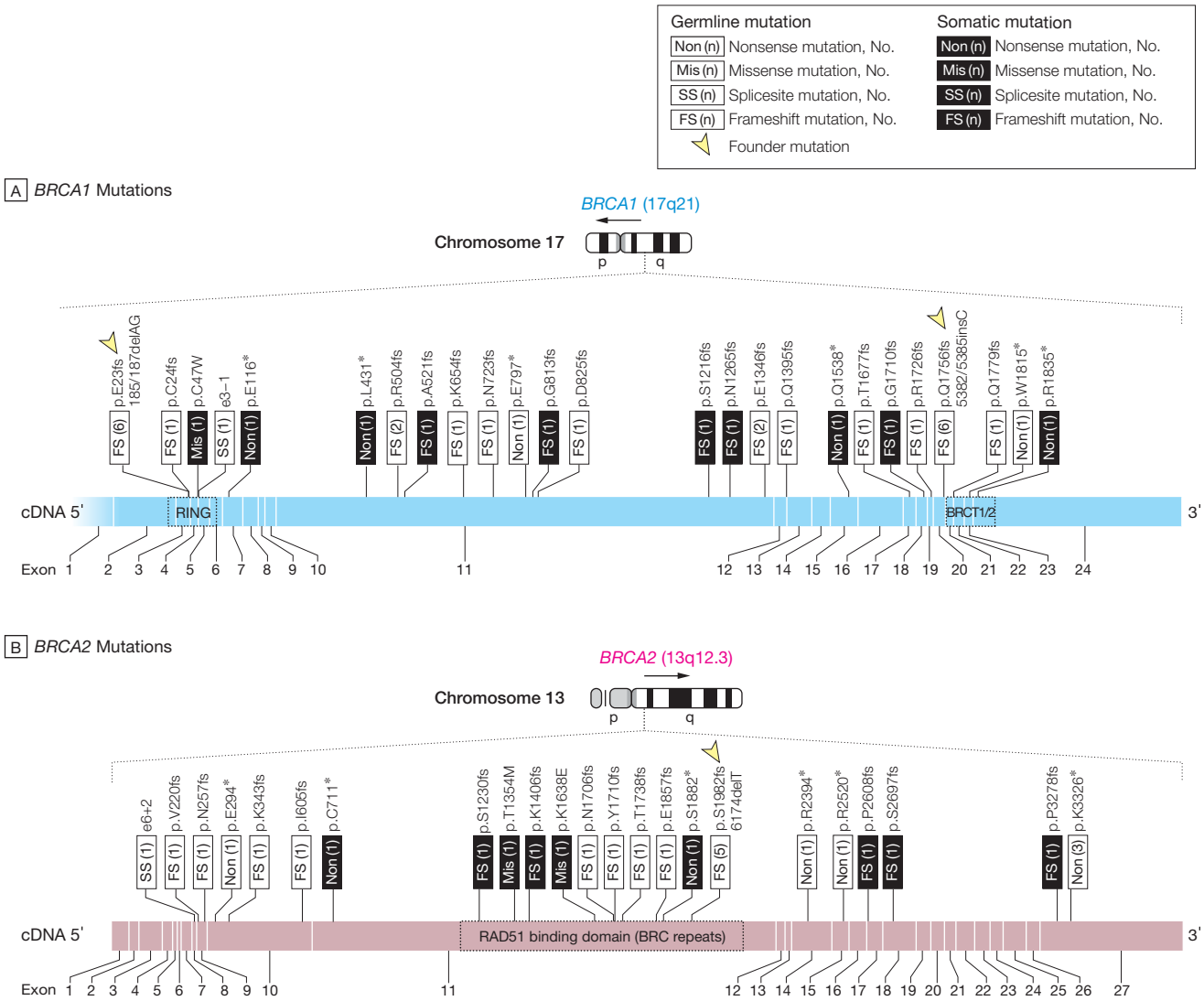
mutated groups. All but 2 *BRCA1* mutations were null mutations (frame shift or nonsense mutations). Among the 37 *BRCA1*-mutated cases, 27 were germ line mutations and 10 were somatic mutations. Twelve of the observed *BRCA1* germ line mutations corresponded to the well-known "founder" mutations, 185/187delAG (E23fs) in the RING-type zinc finger domain and 5382/5385insC (Q1756fs), both of which have been extensively studied in Ashkenazi Jewish populations[21] (FIGURE 1A). Among the 29 *BRCA2*-mutated cases, 20 were germ line mutations and 9 were somatic mutations. Only 5 of the observed *BRCA2* germ line mutations corresponded with the well-known 6174delT (S1982fs) founder mutation[22] (Figure 1B).

Patients with both types of mutations did not differ significantly from each other with respect to tumor stage, grade, or histologic type (Table 1), but patients with *BRCA1* mutations were younger at diagnosis (mean age, 55.9 years) than were those with wild-type *BRCA* (mean age, 61.8 years; $P = .006$; Wilcoxon rank sum test) or *BRCA2* mutation (mean age, 60.9 years; $P = .03$; Wilcoxon rank sum test; Table 1). No differences in OS and PFS duration were observed between germ line and somatic mutations; therefore, these mutation types were pooled for downstream analysis.

Figure 1. *BRCA1/2* Mutations in 316 Ovarian Cancer Cases



BRCT indicates *BRCA1* C-terminal domain; RING, RING-type zinc finger domain. Mutations are mapped to the corresponding exons of *BRCA1* and *BRCA2*.

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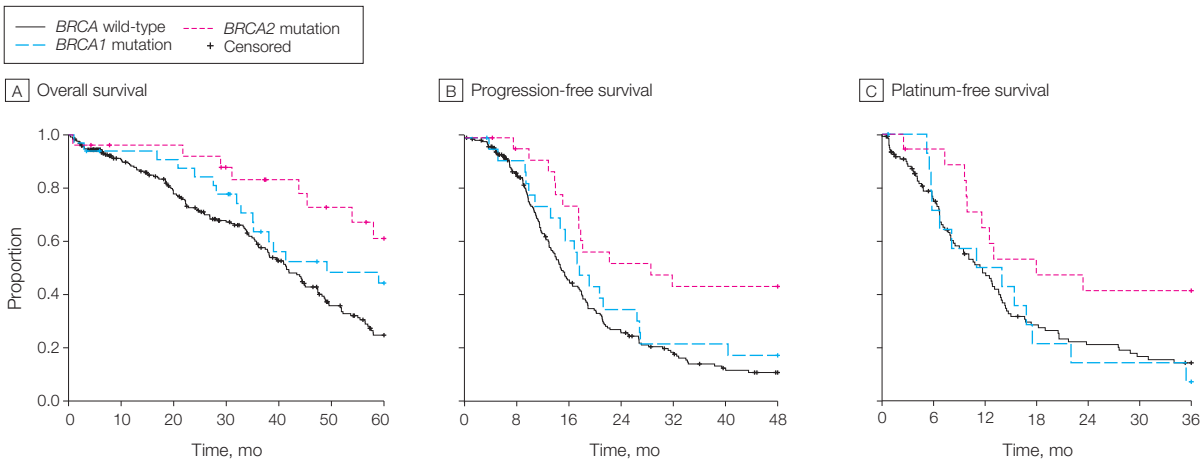
Survival

The 5-year survival rate of *BRCA2* mutation carriers was 61% (95% CI, 43%-87%), which is significantly higher than that of wild-type *BRCA* cases, with 5-year survival rates of 25% in the unadjusted (log-rank $P = .002$; FIGURE 2A) and adjusted ($P = .003$; hazard ratio [HR] = 0.33, 95% CI, 0.16-0.69) models (TABLE 2). In contrast, *BRCA1* mutation carriers had nonsignificant difference in survival compared with wild-type *BRCA* cases in the unadjusted model ($P = .09$; log-rank test; Figure 2A). *BRCA1* mutation carriers' nonsignifi-

Figure 2. Association of *BRCA1/2* Mutations With Survival and Chemotherapy Responses



No. at risk

	A							B							C						
BRCA wild-type	251[a]	189	153	117	82	49	29	209	154	77	42	26	15	10	129	79	47	27	20	14	11
BRCA1 mutation	33	29	28	24	15	12	11	24	21	14	8	5	5	4	15	10	7	3	2	2	1
BRCA2 mutation	26	23	23	19	16	13	10	25	22	17	12	10	10	10	18	16	11	8	7	7	7

A, The log-rank *P* value for *BRCA2*- vs *BRCA1*-mutated cases, .17; for *BRCA1*-mutated vs *BRCA* wild-type cases, .09; for *BRCA2*-mutated vs *BRCA* wild-type, .002. B, The log-rank *P* value for *BRCA2*- vs *BRCA1*-mutated cases, .05; for *BRCA1*-mutated vs *BRCA* wild-type cases, .35; for *BRCA2*-mutated vs *BRCA* wild-type, .001. A and B, The percent probability of survival is plotted vs time since diagnosis in months. C, The percent probability of survival is plotted vs time since the end of adjuvant therapy. The log-rank *P* value for *BRCA2*- vs *BRCA1*-mutated cases, .04; for *BRCA1*-mutated vs *BRCA* wild-type cases, >.99; for *BRCA2*-mutated vs *BRCA* wild-type, .02.
[a] Four of the 314 The Cancer Genome Atlas (TCGA) cases (1 wild-type *BRCA*; 2 *BRCA1* mutations; and 1 *BRCA2* mutation) are not included in the analysis because of missing overall survival data in the TCGA database.

Table 2. Multivariable Models for Overall Survival and Progression-Free Survival in Women With Ovarian Cancer

	Overall Survival				Progression-Free Survival			
	3-Year Rate, % (95% CI)	5-Year Rate, % (95% CI)	HR (95% CI)	*P* Value[a]	3-Year Rate, % (95% CI)	5-Year Rate, % (95% CI)	HR (95% CI)	*P* Value[a]
BRCA status[b]								
BRCA wild-type	58 (51-66)	25 (19-34)	1 [Reference]		16 (11-23)	10 (6-18)	1 [Reference]	
BRCA1 mutation	64 (49-84)	44 (29-67)	0.76 (0.43-1.35)	.35	22 (10-47)	13 (5-38)	0.81 (0.48-1.38)	.44
BRCA2 mutation	83 (69-100)	61 (43-87)	0.33 (0.16-0.69)	.003	44 (27-69)	39 (23-65)	0.40 (0.22-0.74)	.004
BRCA1 methylation	68 (51-89)	24 (11-55)	1.06 (0.62-1.81)	.83	5 (1-32)	NA[c]	1.33 (0.82-2.15)	.24
Tumor stage								
II	92 (79-100)	54 (28-100)	1 [Reference]		56 (33-95)	37 (14-98)	1 [Reference]	
III and IV	60 (54-67)	30 (24-37)	4.12 (1.01-16.74)	.05	16 (12-23)	12 (8-18)	3.31 (1.21-9.06)	.02
Residual tumor, cm								
0[d]	73 (61-88)	49 (34-72)	1 [Reference]		36 (23-55)	22 (11-45)	1 [Reference]	
<1	59 (50-68)	21 (14-31)	1.82 (1.09-3.03)	.02	11 (6-19)	9 (5-17)	1.92 (1.25-2.95)	.003
1-2	61 (39-95)	31 (13-77)	1.42 (0.61-3.28)	.42	15 (2-89)	15 (2-89)	2.29 (0.93-5.64)	.07
>2	47 (34-64)	27 (16-47)	1.93 (1.07-3.50)	.03	9 (3-25)	4 (1-25)	1.72 (1.03-2.87)	.04
Age increase of 1.0 y[e]			1.03 (1.01-1.05)	<.001			1.00 (0.98-1.01)	.88

Abbreviations: HR, hazard ratio; NA, not available.
[a] *P* values were derived from the Cox regression model including all variables in the table.
[b] Two cases with both *BRCA1* and *BRCA2* mutations were excluded from analysis; *BRCA* wild-type cases do not include the *BRCA1* methylation cases.
[c] Not calculable due to lack of data.
[d] Patients with no macroscopic disease are labeled as 0 cm.
[e] Empty cells indicate that data are not applicable for this category.

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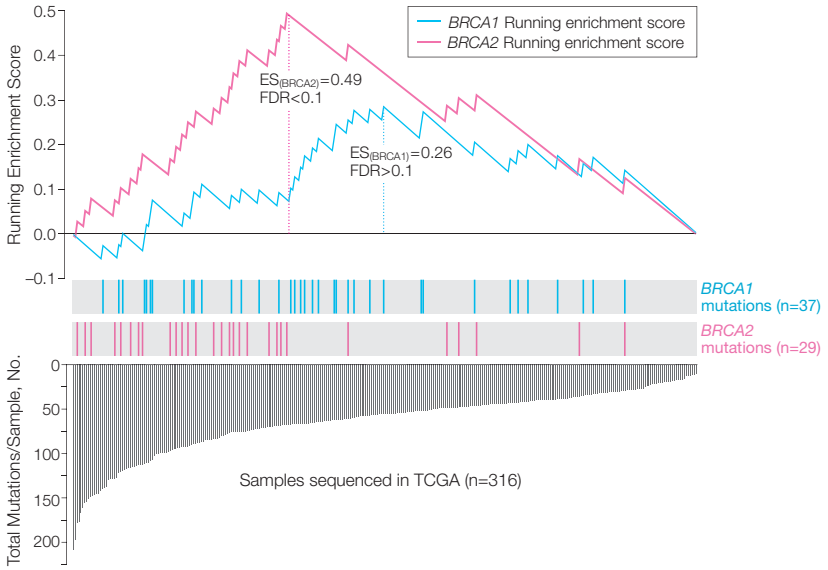
Figure 3. Association of *BRCA1/2* Mutations With Genome Instability



Enrichment score test (ES, detailed in "Methods") of hypermutated sample enrichment in *BRCA1*- and *BRCA2*-mutated cases. Bottom portion shows total numbers of nonsynonymous mutations of 316 decreasingly ranked ovarian cancer cases in The Cancer Genome Atlas (TCGA). The height of each discrete line indicates the number of nonsynonymous mutations in each ovarian cancer case. The middle portion of the plot shows where the samples with *BRCA1* or *BRCA2* mutations appear in the ranked list of samples in the bottom portion. FDR indicates false discovery rate.

cantly longer survival durations vanished when we adjusted for age ($P=.35$; HR, 0.76; 95% CI, 0.43-1.35; Table 2), suggesting that their survival duration was attributable to younger age at diagnosis. *BRCA2* mutation carriers had significantly longer PFS durations than did wild-type *BRCA* carriers ($P=.004$; HR, 0.40; 95% CI, 0.22-0.74; Table 2); no difference was found for *BRCA1* mutation carriers (Figure 2B and Table 2).

A direct comparison between *BRCA1* and *BRCA2* mutation carriers revealed significant difference in PFS between *BRCA1* and *BRCA2* mutation carriers: 44% (95% CI, 27%-69%; Table 2) of *BRCA2*-mutated cases remained progression free 3 years after surgical resection compared with only 22% (95% CI, 10%-47%; Table 2) of *BRCA1*-mutated cases ($P=.05$, log-rank test, Figure 2B).

Responses to Chemotherapy

Among all 316 patients treated with platinum-based adjuvant chemotherapy, 261 experienced primary responses. We determined the association of *BRCA1/2* mutations with chemotherapy response by investigating both primary chemotherapy response and platinum-free duration. Patients who experienced complete or partial responses to adjuvant chemotherapy were defined as primary sensitive, whereas patients with stable or progressive disease during therapy were defined as primary resistant (eFigure 1).

We identified 225 sensitive and 36 resistant cases on the basis of this criterion. Among *BRCA2*-mutated cases, 100% (25 of 25) were primary sensitive compared with 85% (175 of 205) of wild-type *BRCA* cases ($P=.05$; χ^2 test). Only 80% (24 of 30) of *BRCA1*-mutated cases were primary sensitive to platinum-based therapy ($P=.02$ compared with *BRCA2*-mutated cases; χ^2 test).

We next determined the association between *BRCA1/2* mutations and platinum-free duration. As shown in Figure 2C, *BRCA2*-mutated cases had significantly longer platinum-free duration than those with *BRCA1* mutations (log-rank $P=.04$; median platinum-free duration, 18.0 months for *BRCA2*-mutated vs 12.5 months for *BRCA1*-mutated cases) and wild-type *BRCA* cases (log-rank $P=.02$; median platinum-free duration, 11.7 months). There was no difference between *BRCA1* mutation and wild-type *BRCA* cases in platinum-free survival duration. In summary, *BRCA2* mutations were associated with significantly improved primary chemotherapy response and longer platinum-free durations than were *BRCA1*-mutated and wild-type *BRCA* ovarian cancer patients, whereas *BRCA1* mutations had no statistically significant association with primary chemotherapy sensitivity or platinum-free survival compared with wild-type *BRCA* cases.

Mutator Phenotype in Ovarian Cancer Patients With *BRCA2* Mutation

Using whole-exome deep-sequencing data on 316 TCGA cases, we further examined the association between *BRCA1* and *BRCA2* mutations with the mutation rate in the ovarian cancer exome. The enrichment score detailed in Methods was chosen to describe the degree of enrichment of hypermutated ovarian cancer cases in *BRCA1*- and *BRCA2*-mutated patient groups. *BRCA2*-mutated cases were highly enriched with hypermutated samples (enrichment score=0.49; false discovery rate, <0.1; median mutation number per sample, 84 for *BRCA2*-mutated vs 52 for BRCA wild-type cases; FIGURE 3). However, we observed no enrichment of hypermutated samples in *BRCA1*-mutated cases (enrichment score=0.26; false discovery rate, >0.1; Figure 3). We then identified 61 genes that were differentially mutated between *BRCA2* and wild-type BRCA cases ($P<.005$; false discovery rate, <0.2; eFigure 2). A number of these genes are involved in response to DNA damage (eg, *TP63*, *BLM*, and *BCL3;* eTable 1). We could not identify differentially mutated genes between *BRCA1* and wild-type *BRCA* cases using the same criteria.

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Ovarian Cancer Prognosis in *BRCA1* Hypermethylation

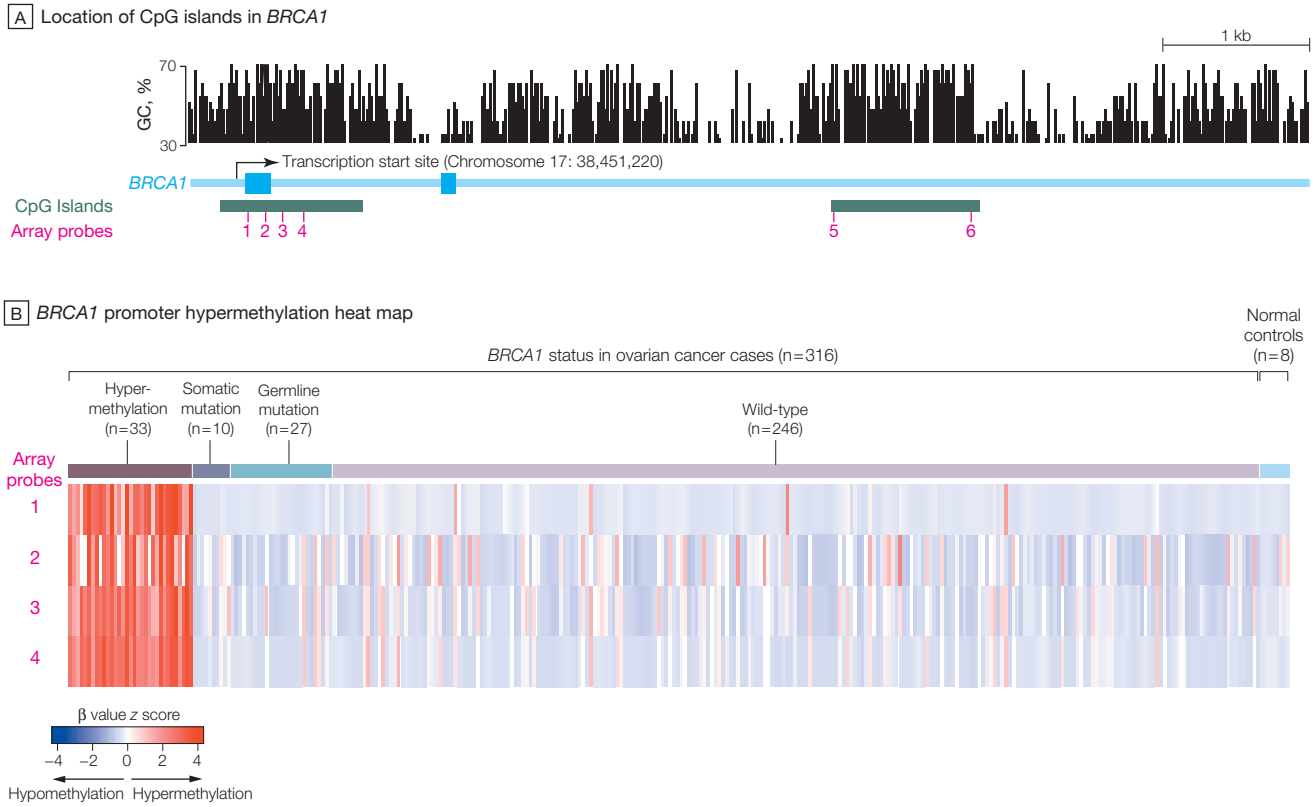
Using the procedures described in Methods, we identified 33 of 316 samples (10.5%) with *BRCA1* inactivation via promoter hypermethylation (FIGURE 4A and Figure 4B). No promoter hypermethylation of *BRCA2* was observed across 316 TCGA samples. The *BRCA1* hypermethylated cases were mutually exclusive with *BRCA1*-mutated cases (Fisher exact test $P=.02$; Figure 4B). *BRCA1* mRNA levels were significantly lower in hypermethylated *BRCA1* cases than in wild-type *BRCA1* cases and normal tissues (Wilcoxon rank sum test $P<.001$; >2-fold change for both comparisons; FIGURE 5), indicating that promoter hypermethylation indeed silenced *BRCA1* expression.

Similar to *BRCA1*-mutated patients, *BRCA1*-hypermethylated patients were significantly younger than wild-type *BRCA* patients ($P=.03$, mean age at diagnosis, 57.3 years for *BRCA1*-hypermethylated vs 61.8 years for *BRCA* wild-type cases; Table 1). *BRCA1*-hypermethylated cases exhibited no significant differences in OS or PFS duration compared with *BRCA* wild-type cases (Table 2) but had significantly shorter durations than those with *BRCA2* mutations (median OS, 86.8 months for *BRCA2*-mutated vs 41.5 months for *BRCA1*-hypermethylated cases; log-rank $P=.01$;and median PFS, 28.6 months for *BRCA2*-mutated vs 14.8 months for *BRCA1*-hypermethylated cases; log-rank $P=.002$; eTable 2). This observation indicates that *BRCA1* inactivation, whether by genomic or epigenomic mechanisms, is not associated with improved ovarian cancer patient outcome.

COMMENT

In this study, an analysis of 316 high-grade serous ovarian cancer cases revealed that only *BRCA2* mutations were an independent predictor of ovarian cancer survival, whereas *BRCA1* mutations were not significantly associated with beneficial OS. In a further analysis, we found no difference in PFS between *BRCA1*-mutated cases and wild-type *BRCA* cases, whereas *BRCA2*-mutated patients had significantly longer PFS durations than did *BRCA1*-mutated and wild-type BRCA patients. Furthermore, using DNA methylation data from the same 316 ovarian cancer cases, we identified 33 *BRCA1* pro-

Figure 4. Hypermethylation of *BRCA1* Promoter in 316 Ovarian Cancer Cases



A. The GC percentage (top panel), CpG islands, and 6 array probes were annotated to *BRCA1* in an Illumina Infinium DNA methylation microarray (Illumina Inc, San Diego, California) and were visualized using the University of California Santa Cruz (UCSC) genome browser. The GC percentage track shows the percentage of G (guanine) and C (cytosine) bases in 5-base windows. Three other probes (see "Methods") annotated to *BRCA1* are not shown because they were too far away from the *BRCA1* transcription start site. B, Heat map shows the DNA methylation of *BRCA1* promoter across 316 cases. Four probes located in the promoter CpG island of *BRCA1* are arranged in rows.

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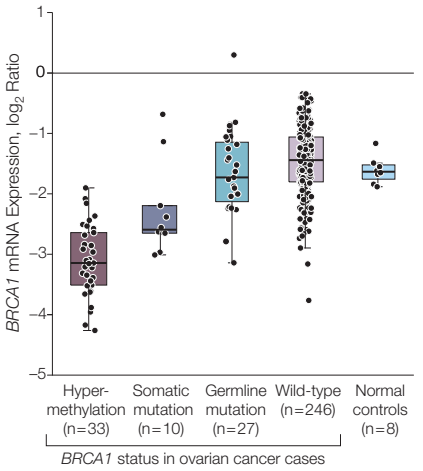
Figure 5. *BRCA1* mRNA Expression in 316 Ovarian Cancer Cases



BRCA1 mRNA expression in different status groups. Each data point represents expression value of *BRCA1* expression in 1 case. The top and bottom of the box indicate lower and upper quartiles; the solid line indicates the median; the whiskers indicate the most extreme data points within the 1.5 times of interquartile range from the box.

moter-hypermethylated cases. Similar to the *BRCA1*-mutated cases, *BRCA1*-hypermethylated cases had similar survival rate to that of wild-type *BRCA* cases but significantly shorter survival relative to *BRCA2*-mutated cases.

Previous studies have mostly combined *BRCA1/2* mutations to assess potential associations with ovarian cancer survival, and some have observed improved outcomes in patients with *BRCA1/2* mutations. These include 4 reports on the association between 3 Ashkenazi Jewish founder *BRCA1/2* mutations and survival of Jewish women with ovarian cancer.[8,10,11,13] Most of these studies are limited by a small sample size and a focus on germ line founder mutations and thus do not have enough statistical power to allow adequate differentiation between the effects of *BRCA1* and *BRCA2* on survival. Our study provides a more representative spectrum of *BRCA* mutations than have previous studies, given that only 7% of patients in the present study were of Ashkenazi Jewish background and 27% of our *BRCA*-positive patients had Jewish founder mutations.

Our analyses of chemotherapy response confirmed our observations regarding survival by demonstrating that all *BRCA2*-mutated cases had significantly higher chemotherapy sensitivity rates and longer platinum-free durations than did *BRCA1*-mutated and wild-type *BRCA* cases. In accordance with our observations for prognosis and chemotherapy response, *BRCA2*-mutated cases, but not *BRCA1*-mutated cases, exhibited a "mutator phenotype" that contained significantly more mutations as determined from whole-exome mutation data. These findings suggest that the different associations between survival and *BRCA1* and *BRCA2* deficiencies likely result from patients' distinct responses to platinum-based treatment, which may be caused by the differing nature of the dysfunction of these 2 genes.

Differences between *BRCA1* and *BRCA2* mutations have been suggested by the results of previous studies. Clinically, although germ line mutations in *BRCA1* and *BRCA2* result in a higher risk for breast and ovarian cancer, carriers of these genes have different risk factors.[1-3] Unlike *BRCA1* mutations, which are almost exclusively associated with female breast and ovarian cancer, *BRCA2* families also have an increased risk for male breast cancer, pancreatic cancer in both males and females, and prostate cancers.[23,24]

Functionally, both *BRCA1* and *BRCA2* have been reported to play key roles in DNA damage repair, but they appear to have distinct but complementary functions.[4,5] The primary function of *BRCA2* appears to be regulation of the RAD51 protein, which is required for double-strand break repair by homologous recombination.[25] It has been established by several research groups that *BRCA2*-mutated cells are recombination deficient and undergo a significantly reduced homology-directed repair of DNA double-strand breaks.[26-28] This explains our observation of a "mutator" phenotype among *BRCA2*-mutated cases and improved chemotherapeutic responses.

In contrast, *BRCA1* plays a more versatile role in tumor suppression through its ability to participate in DNA damage response,[29-32] checkpoint control,[33] mitotic spindle assembly,[34] sister-chromatid decatenation,[35] and centrosome duplication.[36,37] The failure of one of these functions could predispose *BRCA1*-mutated cells to tumorigenesis but not necessarily render the developed cancer cell sensitive to DNA cross-link agents such as cisplatin, as we observed in the present study.

Our observations provide evidence that *BRCA1* and *BRCA2* mutations are differentially associated with patient survival compared with wild-type *BRCA* and that this difference may be a result of distinct response to platinum-based treatment and different associations with genome instability.

However, there are potential limitations in our study. Although, to our knowledge, the patient cohort (316 cases) represents the most comprehensive data composition (both genomic and clinical) assembled, it is still relatively small and our findings should be further validated. In addition, the associations between *BRCA2* mutation and chemotherapy sensitivity and higher-exome mutation rate do not necessarily imply that *BRCA2* mutations affect chemotherapy sensitivity and genome instability. To fully understand and exploit these results, functional studies are required.

Nevertheless, the discovery that *BRCA1* and *BRCA2* deficiencies are associated with differential effects on patient survival and chemotherapy response in ovarian cancer may have important implications for clinical prediction and trial design and sheds new light on the function of these 2 genes.

Author Contributions: Dr Zhang had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Yang and Khan contributed equally to this study.
Study concept and design: Shmulevich, Sood, Zhang.
Acquisition of data: Yang, Khan.
Analysis and interpretation of data: Yang, Khan, Sun, Hess, Shmulevich, Sood, Zhang.
Drafting of the manuscript: Yang, Khan, Sood, Zhang.
Critical revision of the manuscript for important intellectual content: Yang, Khan, Sun, Hess, Shmulevich, Sood, Zhang.

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Statistical analysis: Yang, Khan, Hess.
Obtained funding: Shmulevich, Sood, Zhang.
Administrative, technical, or material support: Sun, Shmulevich.
Study supervision: Shmulevich, Sood, Zhang.

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