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

Da Yang, PhD
Sofia Khan, PhD
Yan Sun, MD, PhD
Kenneth Hess, PhD
Ilya Shmulevich, PhD
Anil K. Sood, MD
Wei Zhang, PhD

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 <.01).

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.

However, conflicting data exist regarding the outcome of BRCA-deficient patients after ovarian cancer develops.

Author Affiliations: Department of Pathology (Drs Yang, Khan, Sun, and Zhang); Department of Bio-statistics (Dr Hess); Department of Gynecologic Oncology and Reproductive Medicine, Department of Cancer Biology, and Center for RNAi and Non-Coding RNA (Dr Sood); University of Texas MD Anderson Cancer Center, Houston; Department of Pathology, Tianjin Medical University Cancer Institute and Hospital, Tianjin, China (Dr Sun); and Institute for Systems Biology, Seattle, Washington (Dr Shmulevich). Dr Khan is now with the Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland.

Corresponding Author: Wei Zhang, PhD, Unit 85, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030 (wzhang@mdanderson.org).

©2011 American Medical Association. All rights reserved.

JAMA, October 12, 2011—Vol 306, No. 14 1557
Corrected on December 20, 2011
clinical course, whereas others have shown the opposite. 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, except for occasional studies reporting improved responses to platinum-based therapy in small cohorts.

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**

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

*Values are reported as No. (%) unless otherwise indicated. Missing values are excluded from the test calculations.

BRCA wild-type cases do not include the BRCA1 methylation cases.

For categorical data (racial/ethnic background, tumor stage and grade, and residual tumor size), the χ² test was used to calculate P values; for age as a continuous variable, the Kruskal-Wallis test was used.

Numbers do not sum because 2 cases with BRCA1 and BRCA2 mutations were excluded.

Residual tumor size is labeled as 0 cm in patients with no macroscopic disease.
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 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 resistant the patient had. Given that suboptimal debulking can contribute to rapid disease progression, 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, 19359 mutations across 316 ovarian cancer samples were downloaded from the TCGA Data Portal. The sequencing and quality control procedures were recently described. 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, 230 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 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 $S_1$, ..., $S_n$, ..., $S_{n+316}$, which are ordered on the basis of total mutations, and a patient group $P$ that contains $G$ members, we defined

$$X_i = \frac{G}{\sqrt{N - G}}$$

if $S_i$ was a member of $P$ and

$$X_i = \frac{\sqrt{N - G}}{G}$$

if $S_i$ was not a member of $P$.

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

$$ES = \max_{1 \leq j \leq N} \sum_{i=1}^{j} X_i$$

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.

Results

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 ($\beta$ values) of 4 probes across 316 samples to separate the epigenetically silenced and nonepigienetically 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 chi-square 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 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 nonsynthetically 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-mutated...
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. 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.

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](image-url)
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

![Graphs showing overall survival, progression-free survival, and platinum-free survival for BRCA wild-type and BRCA mutation cases.](https://example.com/graph2.png)

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.

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

<table>
<thead>
<tr>
<th>BRCA Status(^a)</th>
<th>Overall Survival</th>
<th>Progression-Free Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3-Year Rate, % (95% CI)</td>
<td>5-Year Rate, % (95% CI)</td>
</tr>
<tr>
<td>BRCA2 mutation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA wild-type</td>
<td>58 (51-66)</td>
<td>25 (19-34)</td>
</tr>
<tr>
<td>BRCA1 mutation</td>
<td>64 (49-84)</td>
<td>44 (29-67)</td>
</tr>
<tr>
<td>BRCA2 mutation</td>
<td>83 (69-100)</td>
<td>61 (43-87)</td>
</tr>
<tr>
<td>BRCA1 methylation</td>
<td>68 (51-89)</td>
<td>24 (11-55)</td>
</tr>
</tbody>
</table>

| 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 |                   |                           |             |                     |                   |                           |             |                     |
|                   | 73 (61-88)        | 49 (34-72)               | 1 [Reference] |                           | 36 (23-55)        | 22 (11-45)               | 1 [Reference] |                     |
| <1                | 59 (60-68)        | 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 (0.61-2.83) | .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 yrs\(^a\) | 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.

©2011 American Medical Association. All rights reserved.

JAMA, October 12, 2011—Vol 306, No. 14
Corrected on December 20, 2011

1561
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; \( \chi^2 \) test). Only 80% (24 of 30) of BRCA1-mutated cases were primary sensitive to platinum-based therapy (P = .02 compared with BRCA2-mutated cases; \( \chi^2 \) 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.
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. Location of CpG islands in BRCA1

B. BRCA1 promoter hypermethylation heat map

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.

©2011 American Medical Association. All rights reserved.
BRCA1/2 Mutations in Ovarian Cancer

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.20-28 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.20-22 Checkpoint control,31 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.
Statistical analysis: Yang, Khan, Hess. Obtained funding: Shmulevich, Sood, Zhang. Administrative, technical, or material support: Sun, Shmulevich.

Study supervision: Shmulevich, Sood, Zhang.
Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Drs Hess, Shmulevich, Sood, and Zhang are funded by the National Institutes of Health (NIH) and various foundations and institutional funds. Dr Sun reports no disclosures.

Funding/Support: This study was supported by grants from the NIH (U24CA143835) to Drs Shmulevich and Zhang, from the Blanton-Davis Ovarian Cancer Research Program to Dr Zhang, and the Ovarian Cancer SPORE grant (PS0 CA083639) to Dr Sood. Dr Yang reports being an Odyssey Fellow at MD Anderson Cancer Center. Dr Khan reports being partially supported by an ASLA-Fulbright Research Grant for a junior scholar.

Role of the Sponsor: The NIH, the Blanton-Davis Ovarian Cancer Research Program, the Ovarian Cancer SPORE grant providers, MD Anderson Cancer Center, and the ASLA-Fulbright Research Grant providers had no role in the design or conduct of the study; the collection, analysis, and interpretation of data; or the preparation, review, or approval of the manuscript.

Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official view of the National Institutes of Health.

Online-Only Material: eFigure 1, eFigure 2, eTable 1, and eTable 2 are available at http://www.jama.com.

Additional Contributions: We thank Ann Sutton, a scientific editor in the Department of Scientific Publications of MD Anderson Cancer Center, for editing the manuscript. She received no specific compensation for editing this article.

REFERENCES


