

## Original Investigation

## Inherited Mutations in Women With Ovarian Carcinoma

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**IMPORTANCE** Germline mutations in *BRCA1* and *BRCA2* are relatively common in women with ovarian, fallopian tube, and peritoneal carcinoma (OC) causing a greatly increased lifetime risk of these cancers, but the frequency and relevance of inherited mutations in other genes is less well characterized.

**OBJECTIVE** To determine the frequency and importance of germline mutations in cancer-associated genes in OC.

**DESIGN, SETTING, AND PARTICIPANTS** A study population of 1915 women with OC and available germline DNA were identified from the University of Washington (UW) gynecologic tissue bank (n = 570) and from Gynecologic Oncology Group (GOG) phase III clinical trials 218 (n = 788) and 262 (n = 557). Patients were enrolled at diagnosis and were not selected for age or family history. Germline DNA was sequenced from women with OC using a targeted capture and multiplex sequencing assay.

**MAIN OUTCOMES AND MEASURES** Mutation frequencies in OC were compared with the National Heart, Lung, and Blood Institute GO Exome Sequencing Project (ESP) and the Exome Aggregation Consortium (ExAC). Clinical characteristics and survival were assessed by mutation status.

**RESULTS** Overall, the median (range) age at diagnosis was 60 (28-91) years in patients recruited from UW and 61 (23-87) years in patients recruited from the GOG trials. A higher number of black women were recruited from the GOG trials (4.3% vs 1.4%;  $P = .009$ ); but in patients recruited from UW, there was a higher proportion of fallopian tube carcinomas (13.3% vs 5.7%;  $P < .001$ ); stage I and II disease (14.6% vs 0% [GOG trials were restricted to advanced-stage cancer]); and nonserous carcinomas (29.9% vs 13.1%,  $P < .001$ ). Of 1915 patients, 280 (15%) had mutations in *BRCA1* (n = 182), or *BRCA2* (n = 98), and 8 (0.4%) had mutations in DNA mismatch repair genes. Mutations in *BRIP1* (n = 26), *RAD51C* (n = 11), *RAD51D* (n = 11), *PALB2* (n = 12), and *BARD1* (n = 4) were significantly more common in patients with OC than in the ESP or ExAC, present in 3.3%. Race, histologic subtype, and disease site were not predictive of mutation frequency. Patients with a *BRCA2* mutation from the GOG trials had longer progression-free survival (hazard ratio [HR], 0.60; 95% CI, 0.45-0.79;  $P < .001$ ) and overall survival (HR, 0.39; 95% CI, 0.25-0.60;  $P < .001$ ) compared with those without mutations.

**CONCLUSIONS AND RELEVANCE** Of 1915 patients with OC, 347 (18%) carried pathogenic germline mutations in genes associated with OC risk. *PALB2* and *BARD1* are suspected OC genes and together with established OC genes (*BRCA1*, *BRCA2*, *BRIP1*, *RAD51C*, *RAD51D*, *MSH2*, *MLH1*, *PMS2*, and *MSH6*) bring the total number of genes suspected to cause hereditary OC to 11.

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Ovarian carcinoma (OC) remains the deadliest gynecologic malignancy.<sup>1</sup> Identifying genetic predisposition offers opportunities for cancer prevention. According to previous studies,<sup>2-4</sup> 13% to 18% of OC is associated with germline mutations in *BRCA1* and *BRCA2*. The lifetime risk of developing OC for a woman with a *BRCA2* mutation is approximately 20% and approximately 50% for a *BRCA1* mutation,<sup>5</sup> and risk-reducing salpingo-oophorectomy has been shown to significantly reduce the risk of OC and all-cause mortality.<sup>6,7</sup> Other genes from the *BRCA*-Fanconi anemia pathway such as *BRIP1*, *RAD51C*, and *RAD51D* have been implicated in hereditary OC.<sup>8-13</sup> We previously reported the frequency of these mutations in a small set of unselected patients with OC (n = 360).<sup>4</sup> Several publications<sup>12-14</sup> reporting on a recent series of unselected OC found lower rates of mutations in all OC genes, perhaps secondary to different sequencing methods. Mutations in other genes within this pathway (*PALB2*, *BARD1*, *NBN*, and *CHEK2*, among others) have been identified in patients with OC, but it is unknown if these mutations confer an elevated risk of OC.<sup>4</sup>

We sought to determine the frequency of mutations in known or suspected OC susceptibility genes in a large unselected group of patients with ovarian, peritoneal, and fallopian tube carcinoma (collectively, OC) using a comprehensive targeted sequencing method.

## Methods

Patients with OC were identified from 3 sources: (1) patients undergoing primary treatment at the University of Washington (UW) Medical Center, and (2) patients who consented for translational research with available DNA from Gynecologic Oncology Group (GOG) protocol 218, and (3) GOG protocol 262. Patients were enrolled at diagnosis and were not selected for age or family history. Pathology was centrally reviewed by gynecologic pathologists, and unsure cases were resolved by consensus. GOG-218 and GOG-262 were large randomized phase III trials studying primary advanced-stage OC. A subset of the UW patients has been previously described.<sup>4</sup> All patients provided written informed consent on protocols approved by an institutional review board.

Germline DNA extracted from blood was sequenced using BROCA, a targeted capture, massively parallel sequencing test developed at the University of Washington.<sup>15</sup> For this study, we sequenced *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *FAM175A*, *FANCP*, *MLH1*, *MSH2*, *MSH6*, *MRE11A*, *NBN*, *PALB2*, *PMS2*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, and *TP53*. Sequencing reads were aligned to the human reference genome (hg19). Variants were identified using GATK37 and Pindel after indel realignment and base quality recalibration. Variants including copy number variations (CNVs) were detected as previously described.<sup>15-17</sup> Missense mutations were only included if proven to be damaging (eg, *BRCA1* C61G<sup>18</sup>). *TP53* missense mutations were classified as deleterious based on available functional data as per the International Agency for Research on Cancer.<sup>19</sup>

Two publically available, overlapping, online exome sequencing data sets were used to estimate population muta-

### At a Glance

- The purpose of this study was to determine the frequency and importance of inherited mutations in cancer-associated genes in women with ovarian cancer (OC), unselected by age or family history.
- Of 1915 OC patients, 347 (18%) carried a germline mutation in a gene associated with OC risk.
- Mutations in *BRCA1*, *BRCA2*, *BRIP1*, *RAD51C*, *RAD51D*, *PALB2*, and *BARD1* were more frequent in patients with OC than in the population.
- Mutations were found in all OC histologic subtypes with the exception of mucinous histology, suggesting that genetic testing is warranted in all nonmucinous histologies.
- Mutation status influenced both progression-free and overall survival in OC clinical trials and should be considered when interpreting results and designing future trials.

tion frequencies: the European American (EA) data set from the National, Heart, Lung, and Blood Institute Exome Sequencing Project (ESP)<sup>20</sup> and the Exome Aggregation Consortium (ExAC).<sup>21</sup> To account for inaccuracy in indel calling in the ESP, we visually inspected the read data for all coding indel calls in the ESP data set for the genes of interest. Calls that were low quality (<17 Phred score) and low coverage (<5) were excluded. The EA group was used because most OC subjects were white, and the total ESP population overrepresents African Americans. ExAC mutation frequencies were weighted to match the racial distribution in OC cases. Because these exome databases do not include CNVs, CNVs were removed from the OC frequencies for this comparison. Splice mutations were also excluded from OC and population mutation frequencies because they could not all be verified to be damaging.

Clinical information was collected as per the GOG 218 and 262 protocols or extracted from the medical records for UW patients. Race and ethnicity (self-reported in the GOG patients and as noted in the medical records for UW patients) were assessed to determine if they affected mutation rates.

Contingency tables were analyzed with  $\chi^2$  or Fisher exact tests. In GOG patients, progression-free survival (PFS) and overall survival (OS) were defined as the time between enrollment and progression<sup>22</sup> or death, respectively. Proportional hazards models were used to provide estimates of relative hazards adjusted for clinical characteristics (further description, as well as the log files of the statistical analyses, are available in the Supplement). The Wald test was used to assess the null hypotheses of equal hazards. All *P* values are 2-sided.

## Results

### Description of Study Population

Clinical characteristics are provided in Table 1. The median (range) age at diagnosis was 60 (28-91) years for UW patients and 61 (2387) years for GOG patients. There was an increased fraction of black women recruited from the GOG trials (4.3% vs 1.4%; *P* = .009); but in patients recruited from UW, there was a higher proportion of fallopian tube carcinomas (13.3% vs 5.7%; *P* < .001); stage I and II disease (14.6% vs 0% [GOG trials

Table 1. Clinical Characteristics of Ovarian Cancer Patients

Characteristic	No. (%)	
	GOG 218 and 262	UW
No.	1345	570
Age, y		
<40	35 (2.6)	16 (2.8)
40-49	176 (13.1)	90 (15.8)
50-59	426 (31.7)	165 (28.9)
60-69	451 (33.5)	172 (30.2)
70-79	229 (17.0)	92 (16.1)
≥80	28 (2.1)	35 (6.1)
Race/ethnicity		
Non-Hispanic white	1176 (87.4)	505 (88.6)
Hispanic	53 (4.3)	16 (2.8)
Non-Hispanic black	58 (4.3)	8 (1.4)
Asian/Pacific Islander	27 (2.0)	11 (1.9)
Other or unknown	31 (2.3)	30 (5.3)
Disease site		
Ovary	1076 (80.0)	426 (74.7)
Peritoneal	192 (14.3)	57 (10.0)
Fallopian tube	77 (5.7)	76 (13.3)
Ovary/endometrial	0	11 (1.9)
Stage <sup>a</sup>		
I	0	41 (7.2)
II	0	42 (7.4)
III	977 (72.6)	380 (66.7)
IV	365 (27.1)	107 (18.8)
Histology		
Serous		
High-grade	1118 (83.1)	380 (66.7)
Low-grade	51 (3.8)	19 (3.3)
Carcinoma, NOS	84 (6.2)	81 (14.2)
Endometrioid		
Low-grade	4 (0.3)	9 (1.6)
High-grade	38 (2.8)	26 (4.6)
Clear cell	30 (2.2)	28 (4.9)
Carcinosarcoma	3 (0.2)	19 (3.3)
Mucinous	9 (0.7)	7 (1.2)
Transitional cell	8 (0.6)	1 (0.2)

Abbreviations: GOG, Gynecologic Oncology Group; NOS, not otherwise specified; UW, University of Washington.

<sup>a</sup> Stage was not available for 3 GOG patients.

were restricted to advanced-stage cancer]); and nonserous carcinomas (29.9% vs 13.1%,  $P < .001$ ).

### Frequency of Mutations in *BRCA1*, *BRCA2*, and Mismatch-Repair Genes

Of 1915 patients, 182 (9.5%) had mutations in *BRCA1* and 98 (5.1%) had mutations in *BRCA2*. Of *BRCA1* and *BRCA2* mutations, 38 of 280 (13.6%) were in Ashkenazi Jewish founder mutations *BRCA1* 185delAG (c.68\_69delAG) ( $n = 18$ ) and 5382insC (c.5266dupC) ( $n = 14$ ) and *BRCA2* 6174delT (c.5946delT) ( $n = 6$ ). Overall, 16 of 182 (8.8%) *BRCA1* mutations were large genomic duplications or deletions, also called copy number vari-

ants (CNVs). Eight of 1915 patients (0.4%) carried mutations in genes involved in mismatch repair (MMR), including 4 in *PMS2*, 3 in *MSH6*, and 1 in *MLH1*. There were no mutations found in *MSH2*. Mutation frequencies for *BRCA1*, *BRCA2*, and MMR did not differ by ascertainment. Median coverage was 219 fold.

### Mutations in Other Genes and Comparison With Population Frequency

Mutations in *BRCA1*, *BRCA2*, *BRIPI*, *PALB2*, *RAD51C*, *RAD51D*, and *BARD1* were all significantly more common in women with OC compared with the ESP or ExAC (Table 2), and using these data, we categorized these genes together with the MMR genes as OC-associated genes. Other putative cancer-associated genes such as *CHEK2*, *NBN*, *RAD50*, *FAM175A*, and *MRE11A* were not more frequently mutated in women with OC. Deleterious mutations in *ATM* and *TP53* were more frequent in OC patients when compared with ExAC, but this was not significant when compared with the ESP. Odds ratios for OC are presented in Table 2. There were no mutations found in *PTEN* or *FANCP*.

In total, 347 women with OC (18.1%) had 352 mutations in OC-associated genes: 280 (14.6%) in *BRCA1* or *BRCA2*, 64 (3.3%) in another BRCA-Fanconi anemia OC-associated gene (*BRIPI*, *PALB2*, *RAD51C*, *RAD51D*, or *BARD1*), and 8 (0.4%) in an MMR gene. Five patients (1.4%) of 347 had more than 1 mutation. Individual mutations and associated clinical data for OC-associated genes other than *BRCA1* and *BRCA2* are provided in the eTable in the Supplement.

### Clinical Characteristics and Mutation Status

Ovarian cancer-associated mutations were separated into 5 categories: (1) *BRCA1*, (2) *BRCA2*, (3) other BRCA-Fanconi anemia OC-associated (*BRIPI*, *PALB2*, *RAD51C*, *RAD51D*, *BARD1*), (4) mismatch repair (*MSH6*, *PMS2*, *MSH2*, *MLH1*), and (5) no mutation (either a mutation in a gene not clearly associated with OC or no mutation). Women with *BRCA1* mutations had a median (range) age at OC onset of 52 (27-77) years, while the median (range) age at OC onset for those with no mutation was 62 (23-91) years ( $P < .001$ ). The median (range) age of patients with a *BRCA2* mutation was 59 (41-83) years; BRCA-Fanconi anemia OC-associated mutation, 60 (34-79) years; and MMR gene mutations, 50 (47-69) years. Table 3 summarizes clinical information by each OC-associated gene. In the 54 of 570 (9.5%) UW patients with a previous history of breast cancer, 28 of 54 (51.8%) had mutations in OC-associated genes, 17 (31.5%) in *BRCA1*, 9 (16.7%) in *BRCA2*, and 2 (3.7%) in other OC-associated genes (1, *BRIPI* and 1, *RAD51D*).

Figure 1 summarizes mutation frequency by clinical characteristics. Disease site, race, and ethnicity were not associated with mutation frequency. Overall, 294 patients with high-grade (grade 2-3) serous carcinomas had OC-associated gene mutations, an overall mutation frequency of 19.6% and a combined *BRCA1* and *BRCA2* frequency of 16.1%. Patients with unspecified carcinomas, endometrioid, carcinosarcoma, and transitional cell histology had similar mutation rates to high-grade serous histology, both for all OC genes and for *BRCA1* and *BRCA2*. Patients with clear cell histology had a lower overall mutation frequency compared with high-grade serous cases

Table 2. Mutation Frequencies in Ovarian Cancer Cases Compared With Population

Gene	Mutation (Freq)			Cases vs ESP EA, OR (95% CI)	P Value	Mutation (Frequency)		
	Cases (n = 1915)	Minus CNVs/Splice (n = 1915)	Adjusted ESP EA (n = 4300)			ExAC (n = 36 276) <sup>a</sup>	Cases vs ExAC, OR (95% CI)	P Value
<b>Genes More Frequently Mutated in Ovarian Cancer</b>								
<i>BRCA1</i>	182 (0.0950)	160 (0.0836)	8 (0.0019)	48.9 (24.0-100)	<.001	114 (0.0031)	29.0 (22.7-37.1)	<.001
<i>BRCA2</i>	98 (0.0512)	95 (0.0496)	16 (0.0037)	14.0 (8.2-23.8)	<.001	149 (0.0041)	12.7 (9.7-16.4)	<.001
<i>BRIP1</i>	26 (0.0136)	20 (0.0104)	5 (0.0012)	9.1 (3.4-24.2)	<.001	60 (0.0017)	6.4 (3.8-10.6)	<.001
<i>PALB2</i>	12 (0.0062)	9 (0.0047)	2 (0.0005)	10.2 (2.2-47.0)	<.001	39 (0.0011)	4.4 (2.1-9.1)	<.001
<i>RAD51C</i>	11 (0.0057)	7 (0.0037)	1 (0.0002)	15.8 (1.9-128)	.002	39 (0.0011)	3.4 (1.5-7.6)	.005
<i>RAD51D</i>	11 (0.0057)	8 (0.0042)	2 (0.0005)	9.0 (1.9-42.5)	.002	14 (0.0004)	10.9 (4.6-26.0)	<.001
<i>BARD1</i>	4 (0.0021)	4 (0.0021)	0	20.3 (1.1-377)	.009	18 (0.0005)	4.2 (1.4-12.5)	.02
<b>Other Known or Suspected Cancer Associated Genes</b>								
<i>CHEK2</i>	11 (0.0057)	7 (0.0036)	25 (0.0058)	0.6 (0.3-1.5)	.37	297 (0.0082)	0.4 (0.2-0.9)	.04
<i>ATM</i>	11 (0.0057)	10 (0.0052)	9 (0.0021)	2.5 (1.0-6.2)	.07	79 (0.0022)	2.4 (1.2-4.7)	.01
<i>NBN</i>	9 (0.0047)	6 (0.0031)	6 (0.0014)	2.2 (0.7-7.0)	.26	49 (0.0014)	2.3 (0.99-5.4)	.09
<i>TP53</i>	6 (0.0031)	6 (0.0031)	4 (0.0009)	3.4 (0.95-12.0)	.08	39 (0.0011)	2.9 (1.2-6.9)	.03
<i>RAD50</i>	3 (0.0016)	3 (0.0016)	11 (0.0026)	0.6 (0.2-2.2)	.57	87 (0.0024)	0.7 (0.2-2.1)	.63
<i>FAM175A</i>	3 (0.0016)	3 (0.0016)	5 (0.0012)	1.3 (0.3-5.6)	.71	30 (0.0008)	1.9 (0.6-6.2)	.23
<i>MRE11A</i>	2 (0.0010)	1 (0.0005)	1 (0.0002)	2.2 (0.1-36.0)	.52	25 (0.0007)	0.8 (0.1-5.6)	>.99

Abbreviations: CNV, copy number variant; EA, the European American; ExAC, Exome Aggregation Consortium; ESP, Exome Sequencing Project; OR, odds ratio.

<sup>a</sup> Mutation numbers in ExAC are adjusted for racial groups using weighted frequencies.

Table 3. Clinical Characteristics by Ovarian Cancer-Associated Gene<sup>a</sup>

Gene	No.	Age, Median (range)	Stage <sup>b</sup>				Histology								
			I	II	III	IV	HGS	LGS	HGE	LGE	Carc	CC	CS	M	T
<i>BRCA1</i>	182	52 (27-77)	1	4	130	47	155	3	4	0	14	4	1		1
<i>BRCA2</i>	98	59 (41-83)	0	1	75	22	85	1	3	0	9	0	0	0	0
<i>BRIP1</i>	26	65.5 (43-79)	1	0	12	13	22	0	0	1	3	0	0	0	0
<i>PALB2</i>	12	56 (49-65)	0	0	10	2	9	0	0	0	2	1	0	0	0
<i>RAD51C</i>	11	64 (47-70)	0	1	7	3	7	0	1	0	2	0	1	0	0
<i>RAD51D</i>	11	54 (34-75)	1	0	5	5	7	0	1	0	3	0	0	0	0
<i>BARD1</i>	4	55.5 (53-60)	0	1	2	1	3	0	0	0	1	0	0	0	0
<i>PMS2</i>	4	52.5 (48-69)	0	0	2	2	4	0	0	0	0	0	0	0	0
<i>MSH6</i>	3	49 (47-62)	2		1	0	1	0	0	2	0	0	0	0	0
<i>MLH1</i>	1	47	0	0	0	1	0	0	0	0	1	0	0	0	0
None	1568	62 (23-91)	37	35	1117	376	1208	66	55	11	131	53	20	16	8

Abbreviations: Carc, unspecified carcinoma; CS, carcinosarcoma; CC, clear cell; HGE, high-grade, grade 2-3, endometrioid; HGS, high-grade, grade 2-3, serous; LGE, low-grade, grade 1, endometrioid; LGS, low-grade, grade 1, serous; M, mucinous; T, transitional cell.

<sup>a</sup> If a patient demonstrated more than 1 mutation (n = 5), each gene mutated is listed.

<sup>b</sup> Stage was not available for 3 GOG patients.

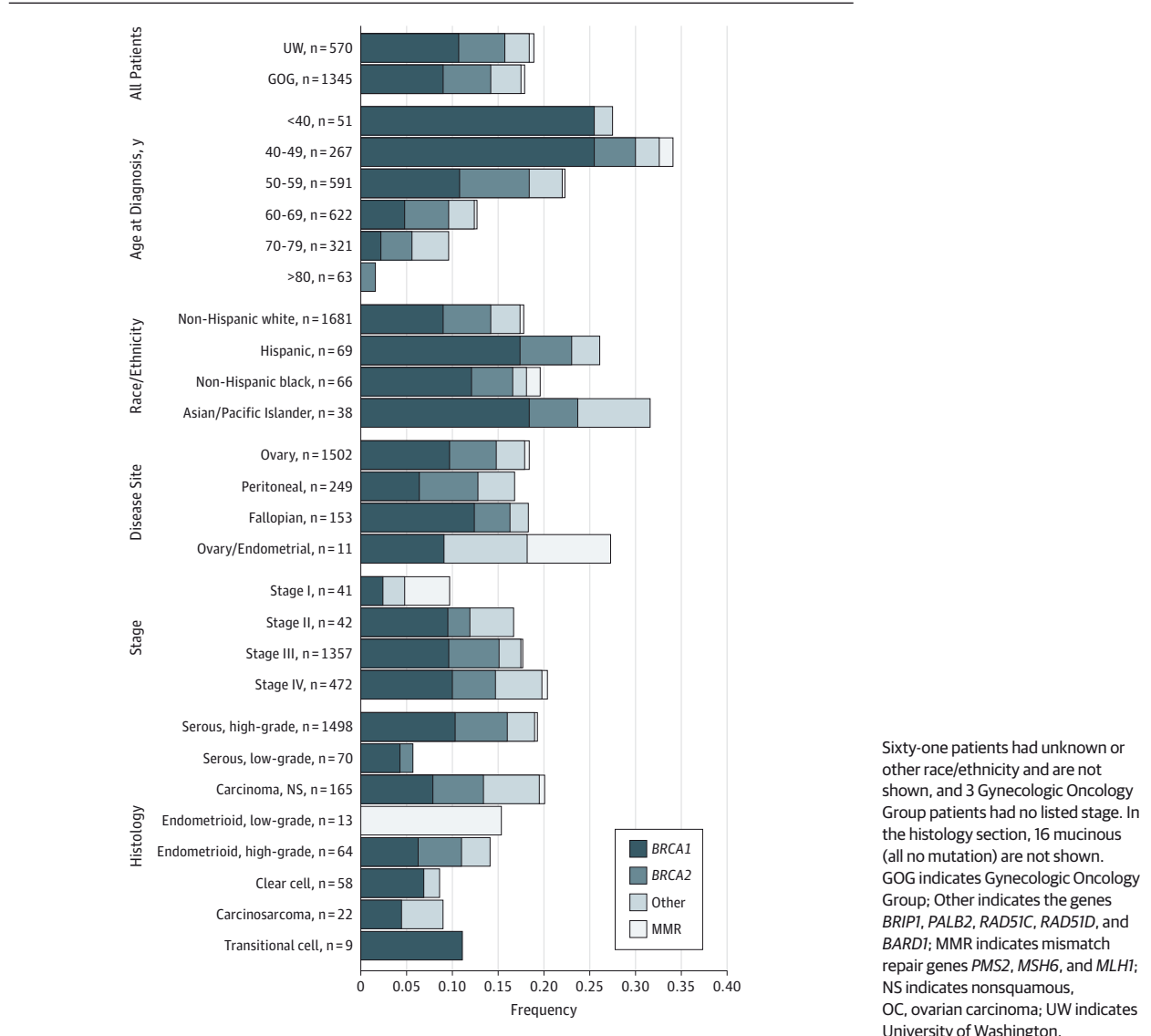
(8.6% vs 19.6%; *P* = .04), but differences in the *BRCA1* and *BRCA2* mutation frequency were not significant (6.9% vs 16.1%; *P* = .07). In patients with low-grade (grade 1) serous histology compared with high-grade serous cases, there were fewer mutations overall (5.7% vs 19.6%; *P* = .003) and for *BRCA1* and *BRCA2* (5.7% vs 16.1%; *P* = .02). In 16 women with mucinous histology, no OC-associated mutations were found.

**Survival**

In the GOG patients, hazard ratios (HRs) for progression and death were adjusted for protocol, study treatment, stage, residual disease, and initial performance status. The GOG pa-

tients were followed for 5 years. The median PFS for GOG patients without a *BRCA2* mutation was 21.9 months, and the median PFS for those with no mutation was 14.2 months (HR, 0.60; 95% CI, 0.45-0.79; *P* < .001). The median OS for GOG patients with a *BRCA2* mutation had not yet been reached, and the median OS for GOG patients with a *BRCA2* mutation was 44 months (HR, 0.39; 95% CI, 0.25-0.60; *P* < .001) (Figure 2). The median OS in patients with a *BRCA1* mutation was 56 months and was intermediate between patients with a *BRCA2* mutation and women without mutations. Relative to patients without mutations, the HR for *BRCA1* mutations was 0.75 (95% CI, 0.56-1.00; *P* = .05) (Figure 2B). In the UW group,

Figure 1. Mutation Status by Clinical Characteristics in Patient With OC

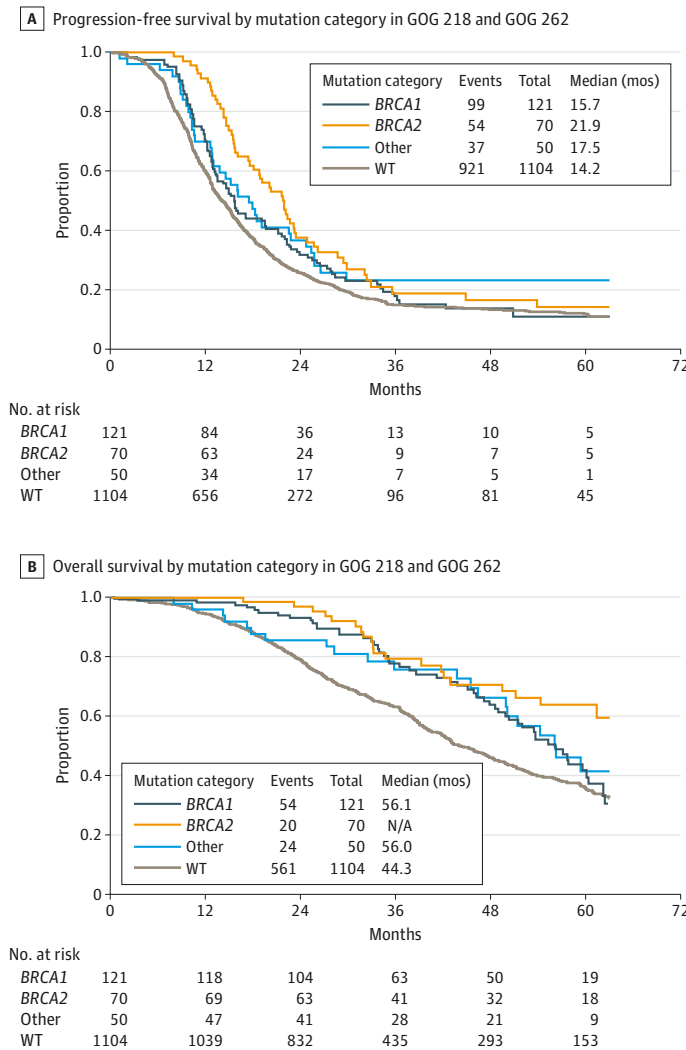


OS for women with stage-III and stage-IV OC was longer in both patients with a *BRCA2* mutation (median, 70 months;  $P = .004$ , log-rank) and *BRCA1* mutation (median, 46 months;  $P = .04$ , log-rank) when compared with women without mutations (median, 36 months). Exploratory analyses of the individuals from the GOG studies suggest that compared with those without mutations, the PFS event rates for those individuals with *BRCA1* ( $P = .009$ ) or *BRCA2* ( $P < .001$ ) mutations are time-dependent (Figure 2A). Specifically, the PFS event rate is initially lower for those with *BRCA1* or *BRCA2* mutations but this advantage declines over time. There is a similar time-dependent decline in the relative death rate for those with *BRCA1* mutations compared with those without mutations ( $P < .001$ ) (Figure 2B). In both GOG (Figure 2) and UW patients (data not shown), survival was similar for women with mutations in *BRCA1* and other OC-associated genes in the BRCA-Fanconi anemia pathway, but the analysis was limited due to low non-*BRCA* mutation frequency.

## Discussion

*BRCA1* and *BRCA2* are part of the BRCA-Fanconi anemia DNA repair pathway that controls DNA repair via homologous recombination.<sup>23-25</sup> It is plausible that damaging mutations in other genes in this pathway may also confer a risk of OC. Indeed, our data demonstrate that mutations in the BRCA-Fanconi anemia OC-associated genes *BRIP1*, *RAD51C*, and *RAD51D* together account for an additional 2.5% of unselected OC. Previous studies of high-risk families or specific founder mutations have suggested that damaging mutations in *BRIP1*, *RAD51C*, and *RAD51D* confer a relative risk increase for OC of 6 to 8 fold and an absolute lifetime risk of 10% to 15%.<sup>8-11,26,27</sup> Our data from unselected patients with OC confirm *BRIP1*, *RAD51C*, and *RAD51D* as important OC-associated genes. The Fanconi anemia gene *BRIP1* (*FANCF*), mutated in 1.4% of women with OC, is the next most commonly mutated gene in OC after *BRCA1* and *BRCA2*.

Figure 2. Survival by Mutation Category in the GOG 218 and GOG 262 Trials



A, Progression-free survival by mutation category in GOG 218 and GOG 262. B, Overall survival by mutation category in GOG 218 and GOG 262. GOG indication Gynecologic Oncology Group; NA indicates not applicable; Other indicates the genes *BRIP1*, *PALB2*, *RAD51C*, *RAD51D*, and *BARD1*; WT indicates wild type.

In addition to identifying mutations in genes already implicated in OC, we identified mutations in the *BRCA*-Fanconi anemia genes *PALB2* and *BARD1* more frequently in women with OC compared with population frequencies in the ESP and ExAC (Table 2). *PALB2* (*FANCN*) is a Fanconi anemia gene whose protein binds *BRCA1* and *BRCA2* at sites of DNA damage.<sup>28</sup> Mutations in *PALB2* are associated with an elevated risk of breast cancer and have been identified in families with both breast cancer and OC<sup>29-31</sup> but have not been clearly associated with OC risk.<sup>28,29</sup> An analysis of germline mutations in high-grade serous OC from the Cancer Genome Atlas project identified *PALB2* as the only gene other than *BRCA1* and *BRCA2* to be significantly more frequently mutated compared with a subset of ESP controls from the Women’s Health Initiative. The *PALB2* mutation frequency in our larger series was significant compared with population rates and was associated with similar odds ratios for OC as *RAD51C*, *RAD51D*, and *BRIP1* (Table 2). The corrected frequency of *PALB2* mutations in the ESP is consistent with *PALB2* mutation rates from other previously published control sets<sup>30,32</sup> where full

sequencing of *PALB2* was performed supporting the use of the ESP as a comparison population.

Three recent publications<sup>12-14</sup> by the Ovarian Cancer Association Consortium (OCAC) have examined rates of mutations in *BRCA1*, *BRCA2*, *MMR* genes, *RAD51C*, *RAD51D*, *BRIP1*, *PALB2*, and *BARD1* compared with controls. The sequencing methodology reported by OCAC is different from our study. As the authors acknowledged, their sequencing methods resulted in relatively low coverage, were unable to detect genomic rearrangements, and were likely to lead to underestimation of mutation frequencies.<sup>12</sup> A recent OCAC study<sup>14</sup> demonstrates a less sensitive sequencing method with a *BRCA1* mutation rate of 3.8% that is significantly lower than our *BRCA1* mutation rate of 9.5% (84 of 2222 vs 182 of 1915;  $P < .001$ , Fisher exact test) and lower than that reported in other population-based series.<sup>2,3</sup> Even if we exclude genomic rearrangements, which OCAC could not detect, our 8.7% *BRCA1* mutation rate remains significantly higher (166 of 1915 vs 84 of 2222;  $P < .001$ , Fisher exact test). Despite a mutation rate for *RAD51C* and *RAD51D* that was approximately 30% lower than ours, OCAC

identified an odds ratio similar to our calculation for these genes. OCAC did not find a difference in mutation frequency for *PALB2* or *BARD1* in OC cases vs controls,<sup>13</sup> but their lower mutation rates in cases and an unexpectedly high *PALB2* mutation rate in controls affected their power to detect such a difference.

*BARD1* forms a heterodimer with *BRCA1* mediated by their homologous ring finger motifs.<sup>33</sup> This heterodimerization is critical to several tumor suppressor functions of *BRCA1*, and mutations in *BRCA1* that affect binding to *BARD1* are associated with increased cancer risk.<sup>34</sup> *BARD1* mutations were rare in OC patients but significantly more common than in the general population, leading to wide confidence intervals for the generated odds ratio for OC (Table 2). However, the significant *P* value and the shared homology to *BRCA1* support *BARD1* as a rare OC susceptibility gene. These results are interpreted with some caution as 2 of the *BARD1* mutation carriers also had mutations in *BRCA1* (eTable in the Supplement). Mutations in *BARD1* have also been identified in women with triple negative breast cancer, another phenotype associated with *BRCA1* mutations.<sup>35</sup> Additional studies are needed to define the absolute risk of *BARD1* for both breast cancer and OC. The addition of *BARD1* and *PALB2* to other known OC-associated genes (*BRCA1*, *BRCA2*, *RAD51C*, *RAD51D*, *BRIP1*, *PALB2*, *MSH2*, *MLH1*, *MSH6*, and *PMS2*) brings the total suspected hereditary OC genes to 11.

In contrast to *PALB2* and *BARD1*, mutations in other genes in the BRCA-Fanconi anemia pathway previously implicated in breast cancer risk but not OC risk (*CHEK2*, *NBN*, *RAD50*, *FAM175A*, and *MRE11A*) were not more commonly mutated in patients with OC. Mutations in *TP53* that causes early onset breast cancer and Li Fraumeni syndrome and mutations in *ATM* were slightly more frequent in OC than in population controls, which appeared significant when compared with the larger ExAC database. Given that mutations in all of these genes are rare, confidence intervals are wide, and we cannot fully exclude that some of these genes, in particular *ATM* and *TP53*, are associated with some risk for OC. However, a relatively high risk of OC is unlikely.

Mutations in MMR genes (*MSH2*, *MLH1*, *MSH6*, and *PMS2*), which cause Lynch syndrome are often cited as the other major cause of hereditary OC in addition to *BRCA1* and *BRCA2*.<sup>36</sup> However, MMR mutations were infrequent (*n* = 8; 0.4%) in this series. Interestingly, 7 of 8 (88%) MMR mutations in women with OC occurred in *PMS2* or *MSH6*, in contrast to patients with colon cancer, in which *MSH2* and *MLH1* mutations predominate.<sup>37</sup> Notably, 2 of 3 OCs with *MSH6* mutations were endometrioid and low stage, but all 4 *PMS2*-mutated cases were advanced-stage high-grade serous carcinomas. Our data are consistent with a population-based study<sup>38</sup> of 1638 women with invasive OC who were sequenced for mutations in *MLH1*, *MSH2*, and *MSH6*, where 9 of 1638 (0.5%) had mutations (5 of 9 in *MSH6* [*PMS2* not assessed]).

The National Comprehensive Cancer Network recommends genetic testing for all women affected by OC, but some authors have proposed limiting *BRCA1* and *BRCA2* testing to high-grade serous OC and suggested that *BRCA1* and *BRCA2* mutations found in nonserous cases represent pathological

misclassification.<sup>39</sup> In our study, all cases were centrally reviewed by a panel of gynecologic pathologists, minimizing pathological misclassification. The overall mutation rate for high-grade serous histology was not significantly different from the mutation frequency in undifferentiated carcinoma, endometrioid, or carcinosarcoma histologies. While clear cell and low-grade serous OC did have fewer mutations compared with high-grade serous OC, the mutation rates (5 of 58 patients [8.6%] and 4 of 70 patients [5.7%]) are probably still high enough to warrant genetic testing. Therefore, our data do not support the restriction of genetic testing to women with high-grade serous OC. Race and ethnicity also did not predict mutation status and should not be used to guide testing. Consistent with previous studies, OC survival was correlated with mutation status, with *BRCA2* mutation carriers having the longest PFS and OS.<sup>2,40</sup> This study is unique in that cancer treatments were standardized within the confines of clinical trials, reducing potential bias in how mutation status affected survival. Mutation status should be considered when analyzing outcomes of OC clinical trials.

There are currently no published guidelines for managing unaffected women found to have mutations in *BRIP1*, *PALB2*, *RAD51C*, *RAD51D*, and *BARD1*. We found similar odds ratios for OC conferred by damaging mutations in these genes to the previously identified 6- to 8-fold relative risk in *BRIP1*, *RAD51C*, and *RAD51D*<sup>8-11,26,27</sup> and a similar age distribution of OC diagnosis for women with mutations in these genes compared with *BRCA2* (Table 2) (Table 3). If a lifetime risk of 10% to 15% is confirmed for these genes, it would be reasonable to consider a risk-reducing salpingo-oophorectomy by age 45 years.

### Limitations

There are several limitations to this study. This was not a population-based study, and the GOG cases were limited to stage-III and stage-IV cancers. However, all patients were enrolled at the time of diagnosis, which reduces survival bias, and all patients were also unselected for age or family history. The ESP is thought to reflect US population mutation rates as opposed to cancer-free controls because cancer status is not fully characterized. ExAC has the advantage of sequencing data on a large number of people characterized by racial and ethnic group, but ExAC also includes patients with known malignancies such as those in the Cancer Genome Atlas studies. The inclusion of known patients with cancer in ExAC could falsely lower our calculated odds ratio for OC. Ideally, we would use a larger, well-characterized control group with matching age and race and known cancer status. However, it is reassuring that mutation frequencies in the ESP for these genes were similar to previously published controls.<sup>8,9,30,32,41</sup> Further research is needed to clarify the roles of inherited mutations in *PALB2* and *BARD1* in ovarian cancer risk.

### Conclusions

We present data from a large, comprehensively sequenced group of unselected OC patients and provide data to implicate 2 new suspected hereditary OC genes, *BARD1* and *PALB2*.

Overall, 347 of 1915 (18.1%) OC patients had 352 germline mutations in 11 OC genes (*BRCA1*, *BRCA2*, *BRIP1*, *RAD51C*, *RAD51D*, *PALB2*, *BARD1*, *PMS2*, *MSH6*, *MLH1*, and *MSH2*). Mutations were identified in all histologies of OC with the exception of mucinous carcinoma, and 72 of 352 (20.5%) OC-associated mutations occurred in a gene other than *BRCA1* or *BRCA2*.

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