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ORIGINAL REPORT

BRCA Mutation Frequency and Patterns of Treatment Response in *BRCA* Mutation–Positive Women With Ovarian Cancer: A Report From the Australian Ovarian Cancer Study Group

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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ABSTRACT

Purpose
The frequency of *BRCA1* and *BRCA2* germ-line mutations in women with ovarian cancer is unclear; reports vary from 3% to 27%. The impact of germ-line mutation on response requires further investigation to understand its impact on treatment planning and clinical trial design.

Patients and Methods
Women with nonmucinous ovarian carcinoma (n = 1,001) enrolled onto a population-based, case-control study were screened for point mutations and large deletions in both genes. Survival outcomes and responses to multiple lines of chemotherapy were assessed.

Results
Germ-line mutations were found in 14.1% of patients overall, including 16.6% of serous cancer patients (high-grade serous, 22.6%); 44% had no reported family history of breast or ovarian cancer. Patients carrying germ-line mutations had improved rates of progression-free and overall survival. In the relapse setting, patients carrying mutations more frequently responded to both platin- and nonplatin-based regimens than mutation-negative patients, even in patients with early relapse after primary treatment. Mutation-negative patients who responded to multiple cycles of platin-based treatment were more likely to carry somatic *BRCA1/2* mutations.

Conclusion
BRCA mutation status has a major influence on survival in ovarian cancer patients and should be an additional stratification factor in clinical trials. Treatment outcomes in *BRCA1/2* carriers challenge conventional definitions of platin resistance, and mutation status may be able to contribute to decision making and systemic therapy selection in the relapse setting. Our data, together with the advent of poly(ADP-ribose) polymerase inhibitor trials, supports the recommendation that germ-line *BRCA1/2* testing should be offered to all women diagnosed with nonmucinous, ovarian carcinoma, regardless of family history.

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INTRODUCTION

The association between germ-line mutations in *BRCA1* and *BRCA2* and ovarian cancer risk is well established. Although widely believed that germ-line *BRCA* mutations account for between 5% and 10% of all ovarian cancers,[1-6] recent reports suggest this is probably an underestimate.[4,7] Selection for germ-line *BRCA1/2* mutation testing is currently variable both within and across countries, and offered predominantly on family history grounds in the context of determining and managing cancer risks in family members.[8] Germ-line *BRCA* mutations are associated with longer survival rates after ovarian cancer diagnosis and generally favorable response to platin-based therapy.[1,6,9-16] The significant activity of poly(ADP-ribose) polymerase inhibitors in *BRCA* mutation carriers[17-19,20] has also focused attention on *BRCA1/2* testing early in disease management. Despite this, the utility of incorporating a patient's *BRCA1/2* mutation status in treatment planning remains unclear and is not routinely used in the ongoing management of the ovarian cancer patient.

In this article, we evaluate a prospectively ascertained, population-based cohort of 1,001 Australian women diagnosed with ovarian cancer to measure mutation frequency in an unbiased cohort. We also explored the clinical characteristics of mutation carriers and documented the outcome of primary

treatment and response to treatment for relapsed disease compared with noncarriers.

PATIENTS AND METHODS

Patient Cohort

Patients were prospectively recruited between January 2002 and June 2006 to the Australian Ovarian Cancer Study, an Australia-wide population-based, case-control study[21] (Data Supplement). Ascertainment was independent of family history. Family cancer history data were self-reported at study entry. Eligible patients included women, ages 18 to 80 years, newly diagnosed with histologically confirmed invasive epithelial ovarian, peritoneal, or fallopian tube cancer (n = 1,409). Women with mucinous or borderline cancers were ineligible for genotyping. This study was approved by the Human Research Ethics Committees at the Peter MacCallum Cancer Centre, Queensland Institute of Medical Research, and all participating hospitals.

Mutation Testing

Comprehensive germ-line testing was completed in a certified diagnostic pathology laboratory using sequencing and multiplex ligation-dependent probe amplification, outlined in the Data Supplement. Tumor DNA samples were screened for somatic mutations in all coding exons of *BRCA1* and *BRCA2* using high resolution melt analysis (Data Supplement).[22,23]

Clinical and Pathologic Data

Patients' medical records were comprehensively reviewed at 6-month intervals up to 5 years after diagnosis, and then annually.[24] Chemotherapy details (first and subsequent lines of treatment) and assessments, including CA-125 levels and imaging results, were collated. Median follow-up for the cohort was 63.4 months. Date of first progression was determined based on CA-125 levels and imaging results according to the Response Evaluation Criteria in Solid Tumors guidelines modified for ovarian cancer,[25-27] or by clinical examination. Progression-free survival (PFS) was defined as the interval between histologic diagnosis and first progression, death as a result of disease, or last follow-up. Death as a result of nondisease-related causes was not considered in the calculation of PFS. Overall survival (OS) was defined as the interval between histologic diagnosis and the date of death as a result of disease, or last follow-up. A CA-125 response (to treatment at relapse) was defined as at least a 50% reduction in CA-125 from a pretreatment sample, maintained for at least 28 days. Response was evaluable if the pretreatment CA-125 level was at least twice the upper limit of normal.[25-27] Additional details are provided in the Data Supplement.

Histopathology data were abstracted from the diagnostic pathology report of the primary tumor. Cases were reviewed by an expert panel of gynecologic pathologists (Data Supplement). Tumors were considered high grade if rated grade 2 or 3.

Statistical Analyses

Descriptive statistics and comparisons between groups were performed using two-tailed *t*-tests, tests for heterogeneity, or Fisher's exact tests as appropriate (STATA 9; StataCorp, College Station, TX). Samples classified as unknown for any variable were excluded. Survival analyses were performed using Cox proportional hazards regression models (SSPS version 19; SSPS, Chicago, IL). Kaplan-Meier plots were used to visualize survival characteristics.

RESULTS

Clinical Characteristics of Mutation Carriers

The cohort of 1,001 patients is listed in the Data Supplement. Pathogenic *BRCA1/2* mutations were identified in 14.1% of patients (141 mutations in 141 women; 95% CI, 11.9% to 16.3%; Data Supplement); more than half of the mutations were in *BRCA1* (88 of 141 patients; 62.4%). A large proportion of *BRCA2* mutations occurred in the ovarian cancer cluster region (21 of 53 patients; 39.6%). Several large deletions were found, but at a low frequency (*BRCA1*, five of 850 patients tested; *BRCA2*, three of 848 patients tested; 5.7% of identified mutations). Recurrent mutations recognized as Ashkenazi Jewish/Eastern European founder mutations comprised 10.6% of pathogenic mutations (15 of 141 patients), with an overall frequency of 1.5% in our cohort. We identified 119 unclassified variants in 100 women, including 20 in women with a pathogenic mutation (Data Supplement).

Women with pathogenic mutations were more likely to be diagnosed with tumors at an advanced stage (Table 1). A higher proportion of women with serous tumors carried pathogenic mutations (118 of 709 patients; 16.6%) compared with other histologies; increasing to 22.6% in patients diagnosed with high-grade serous cancers (HGSC; 98 of 433 patients). We previously identified four molecular subtypes of HGSC, based on gene expression patterns[24]; germ-line *BRCA* mutations were not associated with specific molecular subtypes of HGSC (Data Supplement).

Pathogenic *BRCA1/2* mutations were identified in 8.4% of women (10 of 119) with endometrioid ovarian carcinoma (EC). Increasingly, high-grade ECs are being reclassified as HGSC.[28] Eight of the *BRCA1/2*-associated ECs were subsequently reclassified as serous or unspecified adenocarcinoma, after immunohistopathology review (Data Supplement). Four *BRCA1* mutations were identified among 63 women initially reported to have clear cell carcinoma (CCC) or mixed CCC/serous ovarian carcinoma. Immunohistopathology review (Data Supplement) and genomic analyses (Data Supplement) indicated that three of these patients were most likely HGSC with focal clear cell alteration.[29] The remaining patient had overlapping features of CCC and serous carcinoma and might represent the recently recognized subgroup of atypical CCC.[30] No *BRCA1/2* mutations were associated with carcinosarcomas (zero of 34 patients). These findings indicate that *BRCA1* and *BRCA2* germ-line mutations are almost exclusively associated with HGSC.

The relationships between carrier status and clinical characteristics are listed in Table 1. Women with a *BRCA1* mutation were younger at diagnosis than those without (mean age, 53.4 years *v* 60.5 years; $P < .0001$). The mean age of women carrying *BRCA2* mutations (59.8 years) was similar to that of those who did not. Age of onset was a strong predictor of *BRCA1/2* mutation status; 22.2% of women diagnosed before age 50 years carried a mutation, compared with 12.1% of those older than 50 years ($P = .001$). Family cancer history was available for 94.2% of women. A minority of the overall cohort (19.4%) had a potentially significant family history (definitions are listed in the Data Supplement). Forty-four percent (95% CI, 35.8% to 52.2%) of mutation-positive women had no potentially significant family history. Having a mother with breast and/or ovarian cancer was a strong predictor of a *BRCA* mutation: 54% of those whose mothers were diagnosed with breast cancer at age 50 years or younger were mutation-positive (12/22; 95% CI, 33.7% to 75.3%). Women carrying a *BRCA1/2* mutation were more likely to develop breast cancer ($P < .001$); 51.5% of women with a prior breast cancer diagnosis were mutation-positive (35 of 68 patients; 95% CI, 39.6% to 63.4%).

Survival Outcomes

Survival analyses were restricted to women with serous tumors. Histologic subtype is significantly associated with survival[31] and we had insufficient numbers of patients in other subtypes for analysis.

Table 1. Clinical Characteristics of the Patient Cohort

Characteristic	Total No. of Patients (N = 1,001)	*BRCA1/2 Mutation Negative (n = 777)*		*BRCA1/2 Mutation Positive (n = 141)*		*BRCA1 Mutation Positive Alone (n = 88)*		*BRCA2 Mutation Positive Alone (n = 53)*		Unclassified Sequence Variants in *BRCA1/2 (n = 83)*		*P*
		No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	
Primary site												.52
Ovary	809	629	77.8	109	13.5	70	8.7	39	4.8	71	8.8	
Peritoneum	152	117	77.0	24	15.8	14	9.2	10	6.6	11	7.2	
Fallopian tube	40	31	77.5	8	20.0	4	10.0	4	10.0	1	2.5	
Subtype												.005
Serous	709	536	75.6	118	16.6	74	10.4	44	6.2	55	7.8	
Clear cell	63	52	82.5	4	6.3	4	6.3	0	—	7	11.1	
Endometrioid	119	98	82.4	10	8.4	7	5.9	3	2.5	11	9.2	
Other	110	91	82.7	9	8.2	3	2.7	6	5.5	10	9.1	
FIGO stage												.07
I	122	101	82.8	8	6.6	4	3.3	4	3.3	13	10.7	
II	74	57	77.0	9	12.2	7	9.5	2	2.7	8	10.8	
III	566	434	76.7	91	16.1	59	10.4	32	5.7	41	7.2	
IV	113	84	74.3	17	15.0	9	8.0	8	7.1	12	10.6	
Not known	126	101	80.2	16	12.7	9	7.1	7	5.6	9	7.1	
Tumor grade												.01
1	50	45	90.0	2	4.0	2	4.0	0	—	3	6.0	
2	168	135	80.4	18	10.7	8	4.8	10	6.0	15	8.9	
3	567	428	75.5	95	16.8	60	10.6	35	6.2	44	7.8	
Not known	216	169	78.2	26	12.0	18	8.3	8	3.7	21	9.7	
Age at diagnosis, years												< .001
Mean	—	60.5		55.8		53.4		59.8		60.4		
Standard deviation	—	10.6		9.4		8.5		9.5		10.2		
Age at diagnosis group, years												< .001
≤ 40	45	35	77.8	7	15.6	6	13.3	1	2.2	3	6.7	
41-50	153	105	68.6	37	24.2	30	19.6	7	4.6	11	7.2	
51-60	346	254	73.4	59	17.1	34	9.8	25	7.2	33	9.5	
≥ 61	457	383	83.8	38	8.3	18	3.9	20	4.4	36	7.9	
Personal breast cancer history												< .001
No breast cancer diagnosis	920	738	80.2	103	11.2	62	6.7	41	4.5	79	8.6	
Previous breast cancer diagnosis	68	29	42.6	35	51.5	23	33.8	12	17.6	4	5.9	
Concurrent breast cancer diagnosis	7	6	85.7	1	14.3	1	14.3	0	—	0	—	
Post breast cancer diagnosis	6	4	66.7	2	33.3	2	33.3	0	—	0	—	
Potentially significant family history												< .001
Yes	194	105	54.1	75	38.7	43	22.2	32	16.5	14	7.2	
No	749	625	83.4	62	8.3	41	5.5	21	2.8	62	8.3	
Not assessable	58	47	81.0	4	6.9	4	6.9	0	—	7	12.1	

(continued on following page)

Table 1. Clinical Characteristics of the Patient Cohort (continued)

Characteristic	Total No. of Patients (N = 1,001)	*BRCA1/2* Mutation Negative (n = 777)		*BRCA1/2* Mutation Positive (n = 141)		*BRCA1* Mutation Positive Alone (n = 88)		*BRCA2* Mutation Positive Alone (n = 53)		Unclassified Sequence Variants in *BRCA1/2* (n = 83)		*P*
		No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	
Mother's cancer type												< .001
Breast	69	37	53.6	24	34.8	12	17.4	12	17.4	8	11.6	
Ovary	22	8	36.4	12	54.5	9	40.9	3	13.6	2	9.1	
Both	3	0	—	3	100	1	33.3	2	66.7	0	—	
Other	152	116	76.3	28	18.4	18	11.8	10	6.6	8	5.3	
None	661	539	81.5	66	10	41	6.2	25	3.8	56	8.5	
Not assessable	94	77	81.9	8	8.5	7	7.4	1	1.1	9	9.6	
Ethnicity												
White	930	723	77.7	130	14.0	79	8.5	51	5.5	77	8.3	.70
Residual disease												.03
Nil macroscopic	372	301	80.9	40	10.8	25	6.7	15	4.0	31	8.3	
≤ 1 cm	253	191	75.5	46	18.2	30	11.9	16	6.3	16	6.3	
> 1 cm	296	223	75.3	45	15.2	29	9.8	16	5.4	28	9.5	
Not known or size not known	80	62	77.5	10	12.5	4	5.0	6	7.5	8	10.0	
Primary treatment												.34
Carboplatin	85	66	77.6	8	9.4	3	3.5	5	5.9	11	12.9	
Carboplatin/paclitaxel	704	537	76.3	105	14.9	67	9.5	38	5.4	62	8.8	
Carboplatin/other*	25	22	88.0	3	12.0	1	4.0	2	8.0	0	—	
Carboplatin/paclitaxel/other†	97	72	74.2	18	18.6	13	13.4	5	5.2	7	7.2	
Cisplatin with or without other‡	5	4	80.0	0	—	0	—	0	—	1	20.0	
No platins§	3	2	66.7	0	—	0	—	0	—	1	33.3	
No primary chemotherapy	82	74	90.2	7	8.5	4	4.8	3	3.7	1	1.2	
Progression-free survival, months												< .196
Median	—	16.2		20.0		19.4		20.0		18.7		
95% CI	—	14.5 to 17.9		16.6 to 23.4		14.8 to 24.0		8.9 to 31.1		13.7 to 23.7		
Overall survival, months												< .031
Median	—	55.5		62.4		62.4		70.1		54.5		
95% CI	—	49.1 to 61.8		47.7 to 77.0		43.8 to 80.9		48.6 to 91.6		37.3 to 71.8		

NOTE. *P* values calculated comparing *BRCA1/2* mutation–positive (combined) and *BRCA1/2* mutation–negative cases; categorical variables were compared using a χ^2 test (STATA 9), except for tumor grade, personal history of breast cancer, mother's cancer type, and primary treatment, which were tested using a Fisher's exact test (STATA 9). Differences in age at diagnosis were tested by a student's *t* test (STATA 9); PFS/OS comparison computed using a log-rank survival analysis (SPSS). A comparison of *BRCA1* and *BRCA2* mutation carriers is presented in the Appendix.

Abbreviations: FU, fluorouracil; OS, overall survival; PFS, progression-free survival.

*Other agents were FU, cyclophosphamide, docetaxel, doxorubicin, epirubicin, gemcitabine, ifosfamide, liposomal doxorubicin, and topotecan.

†Other agents were FU, bevacizumab, cyclophosphamide, docetaxel, doxorubicin, etoposide, gemcitabine, liposomal doxorubicin, methotrexate, topotecan, and not recorded (n = 3).

‡Other agents were cyclophosphamide, doxorubicin, gemcitabine, and paclitaxel.

§Docetaxel or ifosfamide.

Women with unclassified *BRCA* variants were also excluded. In univariate analyses of the remaining patients, lower tumor stage ($P < .001$) and optimal surgical debulking after primary surgery ($P < .001$) were associated with better rates of survival, consistent with previous reports.[32] Univariate analyses are presented in the Data Supplement. In multivariate analyses, *BRCA1/2* mutation status was an independent predictor of better OS and PFS, even after adjusting for age, stage, and debulking (Data Supplement). A prior

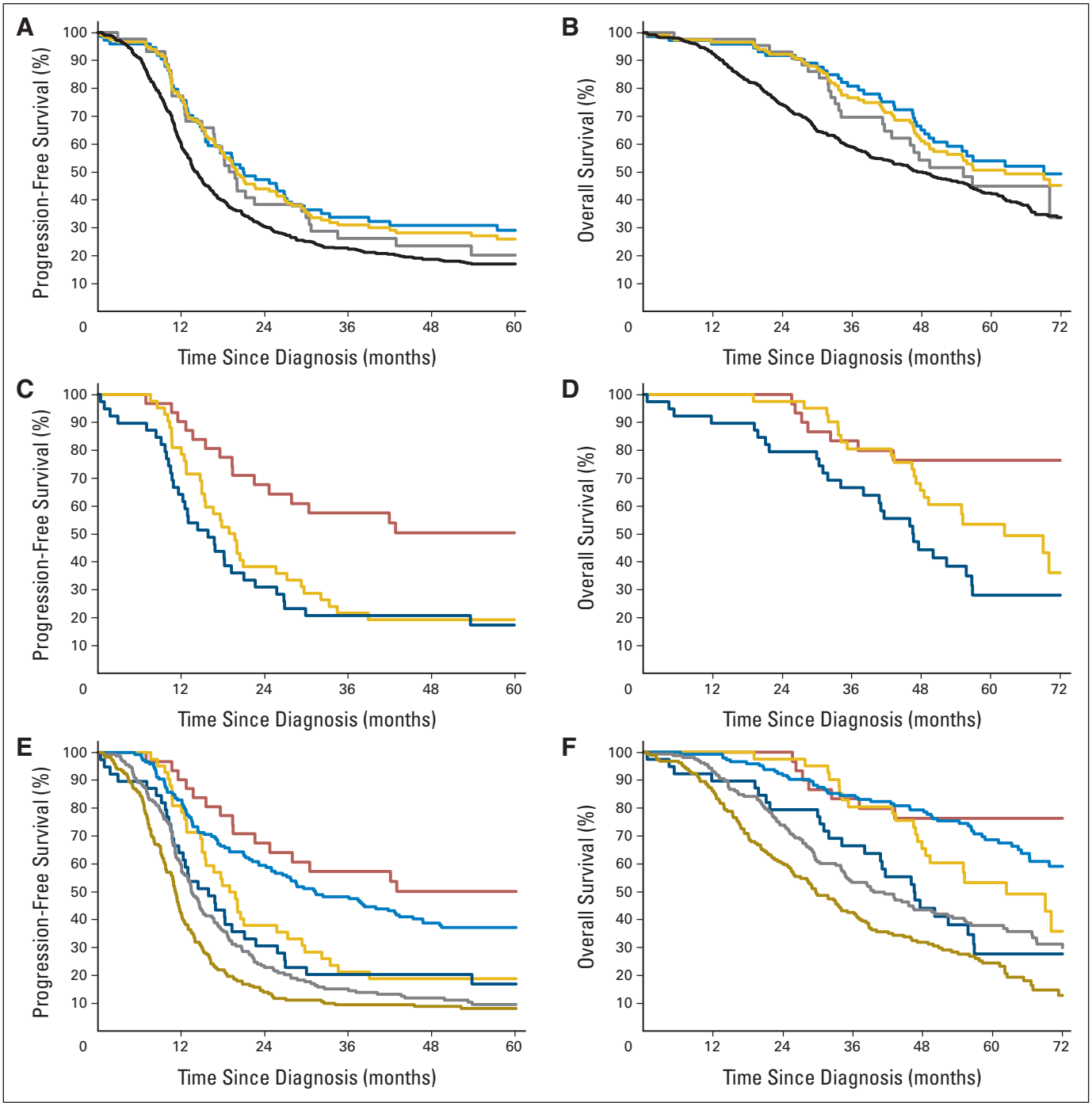

Fig 1. Kaplan-Meier survival analysis; *P* values calculated using a log-rank analysis. Estimated (A) progression-free survival ($P = .01$) and (B) overall survival ($P = .01$) of women with serous tumors by mutation status: *BRCA1* mutation–positive (blue); *BRCA2* mutation–positive (gray); *BRCA1/2* mutation–positive (combined; gold); wild type (black). Death as a result of disease (n = 371); patients who died as a result of nondisease-related causes were censored (n = 40). Estimated (C) progression-free survival ($P < .001$) and (D) overall survival ($P < .001$) of women with serous *BRCA1/2* mutation–positive tumors by amount of residual disease after primary surgery: nil macroscopic disease (red); ≤ 1 cm (gold); > 1 cm (blue). Death as a result of disease (n = 57); patients who died as a result of nondisease-related causes were censored (n = 4). Estimated (E) progression-free survival ($P < .001$) and (F) overall survival ($P < .001$) of women with serous tumors by both mutation status and amount of residual disease: *BRCA1/2* mutation–positive with nil macroscopic disease (red); *BRCA1/2* mutation–positive with ≤ 1 cm residual disease (gold); *BRCA1/2* mutation–positive with > 1 cm macroscopic disease (dark blue); *BRCA1/2* wild-type with nil residual disease (light blue); *BRCA1/2* wild-type with ≤ 1 cm residual disease (gray); *BRCA1/2* wild-type with > 1 cm macroscopic disease (dark gold). Death as a result of disease, n = 348; patients who had died as a result of nondisease-related causes were censored (n = 35).

history of breast cancer did not adversely affect OS after a diagnosis of ovarian cancer.

The improved outcome of mutation-positive women was not universal. For mutation-positive women, late tumor stage and suboptimal tumor debulking were significantly associated with reduced survival in a univariate analysis (Data Supplement). In a multivariate analysis, only the extent of debulking at primary surgery persisted as an independent prognostic factor for survival in patients who were mutation carriers (Data Supplement). Survival outcomes for patients positive for mutations and with nonoptimally debulked disease were similar to patients negative for mutations and with optimally debulked disease (Figs 1E and 1F), for both PFS ($P = .51$) and OS ($P = .85$; log-rank survival analysis; Data Supplement).

Patients with *BRCA1/2* mutations were more likely to have developed visceral metastases (liver, spleen, brain, or lung) within 2 months of first progression (30.4%) than patients not carrying mutations (22.3%, $P = .09$; data not shown). This difference diminished over time. The presence of visceral metastases did not appear to affect OS of patients carrying mutations.

Treatment Response

Responses to second, and subsequent, lines of treatment were compared across all patients, after excluding those with unclassified variants (Fig 2). Of the remaining 918 patients, 837 received chemotherapy during primary treatment. Almost all received a platin-based regimen (835 of 837 patients; 99.8%), most commonly carboplatin/paclitaxel (642 of 835 patients; 76.9%). Patients carrying mutations were less likely to have disease progression within 6 months of the end of primary treatment compared with those not carrying mutations (14.9% compared with 31.7%; $P < .0001$; Figs 2A and 2B). Disease progression within 6 months of completing primary platin-based chemotherapy has conventionally been associated with platinum resistance.[33]

Five hundred one patients received treatment at first progression (Figs 2C to 2J) and of these patients 333 had their first relapse more

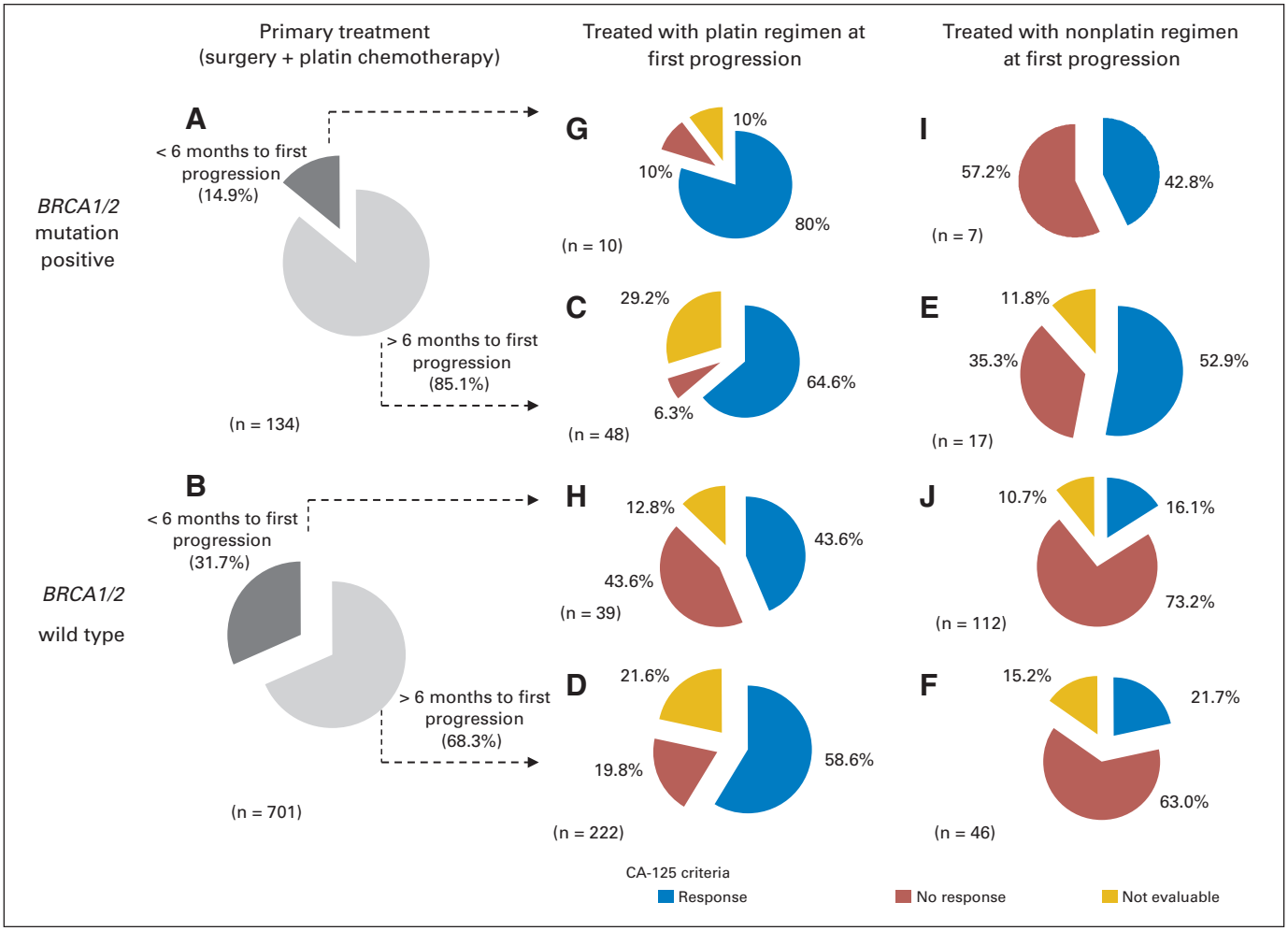

Fig 2. Response to treatment at first progression. Treatment response was based on a 50% decrease in CA-125, maintained for 28 days, as described in the Patients and Methods section. Patients with a sequence variant of unclassified significance were excluded from this analysis. (A) Mutation-positive and (B) mutation-negative patients are divided into those whose disease progressed within 6 months of the end of treatment (dark gray segment) and those whose disease progressed more than 6 months after primary treatment (light gray segment). Response to second line for (C and D) platin-based and (E and F) nonplatin-based treatments in patients with more than 6 months to first progression after primary platinum treatment (n = 333). Response to second line for (G and H) platin-based and (I and J) nonplatin-based treatments in patients whose disease progressed within 6 months of the end of primary platinum treatment (n = 168).

than 6 months after the end of primary treatment (Figs 2C and 2E; Figs 2D and 2F). Among this group, response rates to second-line platin-based chemotherapy were higher in patients carrying mutations than those who did not (64.6% *v* 58.6%; $P = .07$; Figs 2C and 2D). There were also higher response rates to nonplatin-based treatment in a second-line setting in patients with mutations versus those without (52.9% *v* 21.7%; $P = .05$; Figs 2E and 2F).

Using progression within 6 months of the end of primary platin treatment as the threshold, 17 patients carrying mutations could be classified as platinum resistant. Despite this, 10 were re-treated with platin-based chemotherapy at relapse, and of these eight patients (80%) showed a CA-125 response (Fig 2G). Only 17 (43.6%) of 39 patients not carrying mutations who were similarly classified as platinum resistant responded to re-treatment with platin-based chemotherapy (Fig 2H). This was, however, a higher response rate than treatment with a nonplatinum regimen for patients not carrying mutations (18 of 112 patients; 16.1% response; $P = .001$; Fisher's exact test; Fig 2J). The majority of patients who responded to platin-based chemotherapy after a short progression-free interval (< 6 months; 7 of 8 patients positive for mutations and 16 of 17 patients negative for mutations) received conventional doses of platinum at re-treatment, with the remaining two patients receiving a dose-dense platinum schedule. Details of patients treated with platin-based regimens at relapse are listed in Table 2.

We considered factors that may have influenced the clinical decision to re-treat patients with platin-based therapy despite progression within 6 months of completion of primary treatment, including whether those re-treated with platinum were closer to the 6-month cutoff. In both mutation-positive and -negative cohorts, women who were re-treated with platinum had a slightly longer progression-free interval after primary treatment than those given a nonplatin regimen at first progression (median interval: 3.72 months *v* 3.11 months for mutation-negative patients and 5.52 months *v* 3.98 months for mutation-positive patients). Age at diagnosis, International Federation of Gynecology and Obstetrics stage, or optimal debulking status did not appear to influence choice of therapy in the primary resistance setting (data not shown).

Thirty of 134 mutation-positive patients were treated with at least three lines of platin-based chemotherapy, of whom 20 patients (66.7%) had a CA-125 response to third-line treatment. We considered factors that may have maintained sensitivity of some mutation-positive patients to platinum-based treatment. Functional reversion of germ-line *BRCA* mutations can partially restore the open reading frames and contribute to treatment resistance.[34-36] We investigated whether the mutation type or location influenced the time to first progression or death, however, there was no obvious pattern based on mutation location or type (Fig 3). Large deletions may be expected to be more difficult to revert than point mutations, however, none of the 20 patients responding to more than three lines of platinum-based chemotherapy had a large deletion (Data Supplement).

Ninety-eight of 700 mutation-negative patients were treated on a third occasion with platinum and of these 37 patients (37.8%) responded to treatment ($P = .02$; $\chi 2$ test). We investigated possible molecular mechanisms underlying continuing response to platin-based therapies in mutation-negative patients. Fresh-frozen tumor tissue was available for 16 of these 37 women, and these tissues were screened for somatic *BRCA1/2* mutations.[22] Twelve (75%) of 16 tumors were serous and four tumors (25%) had a pathogenic somatic *BRCA* mutation, compared with 6% (eight of 132) unselected HGSC (Alsop et al, manuscript in preparation).

Table 2. Platin-Based Chemotherapy Regimens Administered to Patients Who Relapsed Within 6 Months of Primary Platin-Based Treatment

Treatment	*BRCA1/2* Mutation Positive (n = 10)		*BRCA1/2* Wild Type (n = 39)	
	No. of Patients	%	No. of Patients	%
Platin only				
Carboplatin	4	40	8	20.5
Cisplatin	0	—	8	20.5*†‡§
Platin combination				
Carboplatin/paclitaxel	5	50†‡	4	10.3
Cisplatin/paclitaxel	1	10*†	1	2.6
Carboplatin/docetaxel	0	—	2	5.1
Carboplatin/gemcitabine	0	—	5	12.8
Carboplatin/liposomal doxorubicin	0	—	6	15.4
Carboplatin/etoposide	0	—	1	2.6*†
Cisplatin/etoposide	0	—	1	2.6†‡
Carboplatin/cyclophosphamide	0	—	1	2.6
Cisplatin/doxorubicin/paclitaxel	0	—	1	2.6
Carboplatin/gemcitabine/docetaxel	0	—	1	2.6

*Weekly dosing (one time per week).
†One patient.
‡Weekly (one time per week), dose-dense regimen.
§Three patients.

DISCUSSION

The rate of referral for genetic counseling and testing among ovarian cancer patients is low—6.8% of patients in our series, which is consistent to rates reported in a Canadian series.[37] Even among women with a potentially significant family history in our cohort, only 38.7% had contact with a genetics clinic during their cancer journey. Identification of *BRCA1/2* mutation–positive patients appears to be a lost opportunity, given clear evidence of effective preventative strategies for those patients,[38] in contrast to limited progress in early detection of ovarian cancer[39,40] or improvements in treatment outcomes in advanced disease.[41] Even when genetic testing is performed it usually occurs late in the course of a patient's disease trajectory, yet our study and others[1,6,9,10,12-16] demonstrate that mutation-positive patients have different treatment responses and survival characteristics compared with mutation-negative patients. Collectively, these findings suggest a re-evaluation of the timing and coverage of *BRCA1/2* testing of ovarian cancer patients. We propose that women are routinely referred for genetic counseling and genetic testing either during or soon after their primary systemic therapy is completed, so that this information is available in a timely fashion for inclusion in decisions about subsequent treatment strategies in the event of a relapse.

We identified a germ-line *BRCA1/2* mutation frequency of 14.1% in women with invasive epithelial (nonmucinous) ovarian cancer, increasing to 16.6% in all women diagnosed with serous tumors. This frequency is at the high end of the range historically

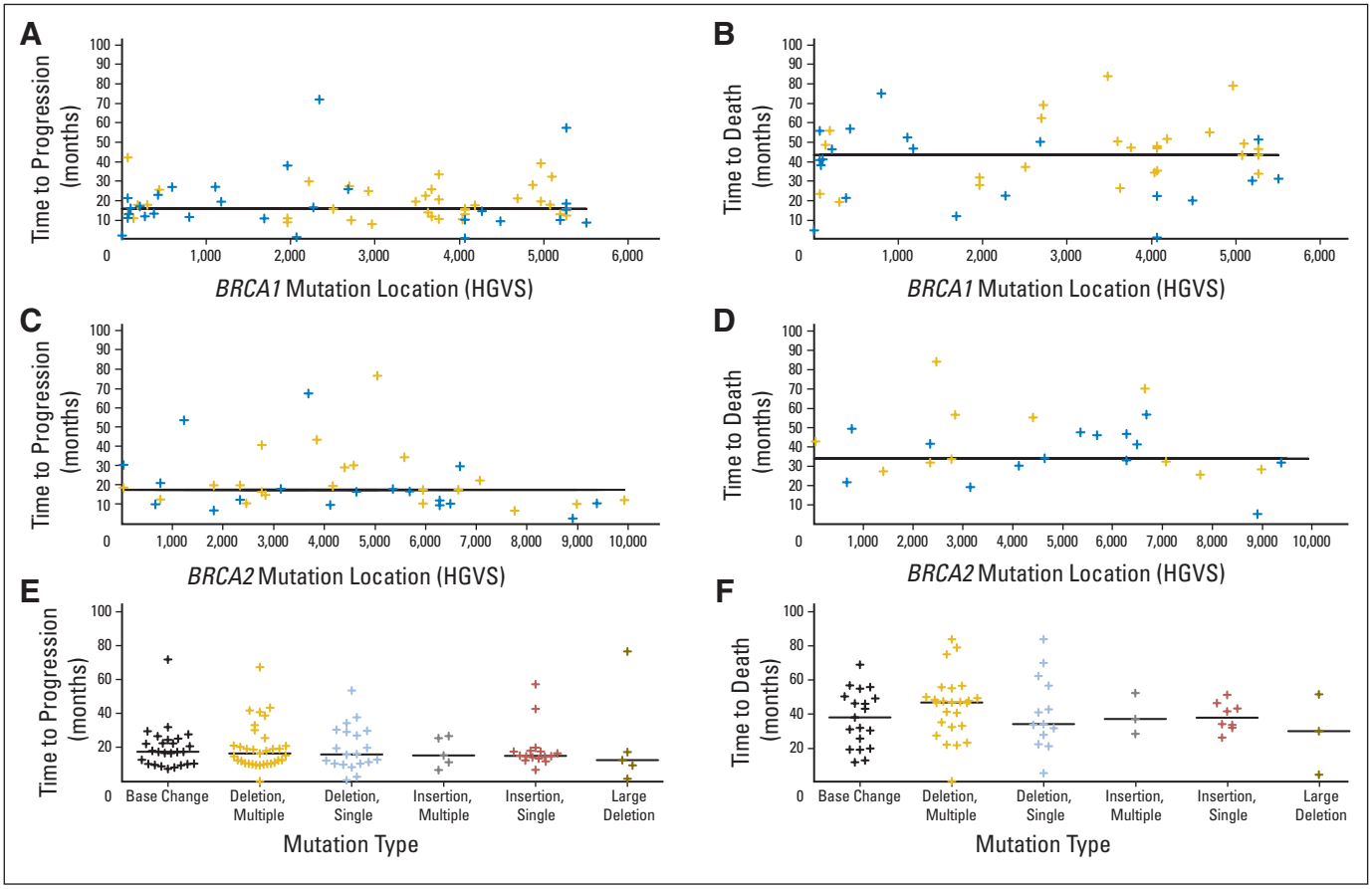

Fig 3. Time to first progression or death was plotted by mutation position along either the *BRCA1* or *BRCA2* gene, or by mutation type. Progression is only plotted for patients whose disease progressed by the censoring date; time to death was only plotted for those patients who died as a result of disease-related causes before the censoring date. The line represents the median. Thirty-five of the mutation-positive patients (24.8%) had remained progression free at the time of data censoring for this study. (A) Time to first progression in patients with a *BRCA1* mutation who were optimally debulked (nil or ≤ 1 cm residual disease; gold plus symbols) or suboptimally debulked (blue) at time of primary surgery. (B) Time to death in *BRCA1* mutation–positive cases who were optimally (gold) or suboptimally (blue) debulked at time of primary surgery. Deaths as a result of disease-related causes (n = 45; plotted); deaths as a result of nondisease-related causes (n = 3; not plotted). (C) Time to first progression in patients with a *BRCA2* mutation who were optimally (gold) or suboptimally (blue) debulked at time of primary surgery. (D) Time to death in *BRCA2* mutation–positive women who were optimally (gold) or suboptimally (blue) debulked at time of primary surgery. Deaths as a result of disease-related causes (n = 26; plotted); deaths as a result of nondisease-related causes (n = 2; not plotted). (E) Time to first progression by mutation type. A deletion or insertion was considered multiple base if at least two base pairs were involved. (F) Time to death by mutation type. Deaths as a result of disease-related causes (n = 71; plotted); deaths as a result of nondisease-related causes (n = 5; not plotted). A deletion or insertion was considered multiple base if at least two base pairs were involved. HGVS, Human Genome Variation Society.

reported,[1-3,5,6] but is consistent with recent findings using more sensitive mutation detection methods.[17,19,39] Our results are not explained by a preponderance of founder mutations in the Australian population. Cohort selection bias has been a concern in some studies,[4,42] and the recent Cancer Genome Atlas analysis of 500 HGSC[7] was not designed as a population-based series. Our study, alongside these selective cohorts, now presents convincing evidence that the *BRCA1/2* germ-line mutation frequency in unselected patients with HGSC is as high as 22%.

At this frequency, *BRCA1/2* mutation screening for all women with nonmucinous invasive ovarian cancer is a highly efficient approach to ascertain new *BRCA1/2* mutation families. Importantly, 44.0% of women identified with mutations in our study did not report a significant family cancer history. Triaging for genetic testing on family cancer history alone can no longer be recommended.[4,6,43] Although our findings suggest that *BRCA1/2* germ-line mutation is essentially associated with HGSC, we also show that routine diagnostic pathologic assessment can lead to some tumors being misclassified. Immunomarkers should improve histotyping of ovarian cancer,[44] however, until this is routine practice is implemented we advise *BRCA1/2* testing of patients with clear cell or high grade endometrioid cancers.

Several studies now demonstrate improved survival outcomes in mutation-positive versus mutation-negative patients[1,9-13,16] and also regarding response to first-line treatment in small series.[14,15] However, often patients received heterogeneous, and potentially outmoded, treatment regimes across long periods of time. The Australian Ovarian Cancer Study cohort was ascertained over a short time period, and detailed first-line and subsequent treatment data were collected. Most patients received contemporary treatment that included a taxane. *BRCA1/2* mutation–positive patients were more likely to have a longer PFS compared with mutation-negative patients. Importantly, we were able to explore treatment responses in the relapse setting. The observation of objective responses to second-line platin therapy in the

mutation-positive patients with early relapse after initial treatment challenges the commonly used definition of platin resistance, ie, progression within 6 months of first-line chemotherapy.[33,45] The high rate of response to platin-based therapy in the recurrent setting argues for continuing use of platin-based regimens at relapse until clear tumor progression on treatment is observed.

We note that 43.6% of mutation-negative women who progressed within 6 months of the end of treatment, and would have been classified as platinum-resistant, also responded to second-line platin-based chemotherapy. We found an enrichment of somatic *BRCA* mutations among patients without a germ-line mutation who repeatedly responded to platin-based treatment, consistent with the improved survival of such women.[7] Similarly, the Cancer Genome Atlas reported that up to 6.3% of HGSC harbor somatic mutations in *BRCA1/2*.[7] These findings highlight the need to evaluate tumors for somatic disruption of the *BRCA* pathway in mutation-negative patients, either by direct mutation testing of relevant genes or using assays to determine the integrity of the DNA homologous repair pathways.[46]

Even among women with the same *BRCA1* or *BRCA2* germ-line mutation there is a range of clinical outcomes, implying other factors exert an additional effect on survival. Mutation positivity does not obviate the need for optimal surgical debulking. Conceivably, the type or genomic position of a germ-line mutation may influence the rate of reversion of mutant alleles[47] although we saw no evidence of this.

In our series, patients with *BRCA1/2* mutation-associated ovarian cancers seemed to be more responsive to cytotoxic chemotherapy, independent of class, compared with mutation-negative patients, consistent with earlier studies that were either clinic-based[14] or included highly selected cases.[7] As the survival advantage of carrier status is of the order sought in trials of new systemic therapies, the generally more favorable response of mutation-positive patients to all classes of treatments and their improved OS, argues strongly for the need to stratify patients based on germ-line *BRCA* mutation status in all new cancer therapy trials.

Germ-line or somatic *BRCA* mutations add to an increased understanding of the determinants of outcome in women with HGSC, including cyclin E amplification,[7,48,49] extent of surgical debulking, and tumor genetic profiles.[24] Our findings suggest changes in the guidelines for genetic testing of all invasive ovarian cancer patients, indicate that the measurement of *BRCA* status should be explicitly integrated into future clinical trial designs as a major stratification factor, and declare *BRCA* status is now ready to be included in the clinical management of women with ovarian cancer. If there is to be an expansion of routine *BRCA* testing to all patients with high-grade ovarian cancer, and its application brought forward into acute clinical management either during or shortly after primary systemic therapy chemotherapy, new streamlined approaches to delivery of genetic counseling and genetic testing will be required.[50]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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