Published in final edited form as:

Gynecol Oncol. 2013 March ; 128(3): 483–487. doi:10.1016/j.ygyno.2012.12.015.

Characteristics of Women with Ovarian Carcinoma who have *BRCA1* and *BRCA2* Mutations not Identified by Clinical Testing

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Abstract

Goals—Few studies have comprehensively tested all ovarian cancer patients for *BRCA1* and *BRCA2* (*BRCA1/2*) mutations. We sought to determine if clinically identified mutation carriers differed in clinical characteristics and outcomes from mutation carriers not identified during routine clinical care.

Methods—We included women with ovarian, tubal or peritoneal carcinoma. BROCA, an assay using targeted capture and massively parallel sequencing was used to identify mutations in *BRCA1/2* and 19 other tumor suppressor genes. We identified subjects with *BRCA1/2* mutations using BROCA that had not previously received standard genetic testing (BROCA, n = 37) and compared them to subjects with *BRCA1/2* mutations identified during routine clinical care (known, n = 70), and to those wildtype for 21 genes using BROCA (wildtype, n = 291).

Results—BROCA mutation carriers were older than known carriers, median age of 58 (range 41 - 77), vs. 51 (range 33-76, p=0.003, Mann-Whitney). 58/70 (82.9%) of known carriers had a strong family history, compared with 15/37 (40.5%) of BROCA carriers, p<0.0001, (Fisher's Exact). Median overall survival was significantly worse for BROCA mutation carriers compared to known mutation carriers, (45 vs. 93 months, p < 0.0001, HR 3.47 (1.79 – 6.72), Log-rank test). The improved survival for *BRCA1/2* mutation carriers (known and BROCA) compared with wildtype cases (69 vs. 44 months, p=0.0001, HR 0.58 (0.43 – 0.77), Log-rank test) was driven by known mutation carriers.

Conclusions—Older age, absence of a strong family history, and poor survival are all associated with decreased clinical identification of inherited *BRCA1/2* mutations in women with ovarian cancer. Using age and family history to direct genetic testing will miss a significant percentage of mutation carriers. Testing should be initiated at the time of diagnosis to maximize identification of mutations and minimize survival bias.

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Conflict of Interest Statement: There are no conflicts of interest to report.

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Keywords

BRCA1; BRCA2; BROCA; genetic testing; ovarian cancer

Introduction

Germline *BRCA1* and *BRCA2* (*BRCA1/2*) mutations are present in approximately 15% of unselected women with ovarian carcinoma (1-3). Women with deleterious *BRCA1/2* mutations have a 20-50% lifetime risk of developing ovarian carcinoma (4), which are generally high grade, high stage, and of serous histology (5-7). The median age of onset of ovarian carcinoma in *BRCA1* mutation carriers (48-53 years) is approximately 10 years earlier than non-hereditary cases. However, *BRCA2* mutation carriers have a similar age of onset as women without *BRCA1/2* mutations (3, 7, 8).

Identification of *BRCA1/2* mutations in women with ovarian carcinoma offers several advantages. Unaffected family members can be offered testing to clarify risk and tailor preventative measures for those with elevated cancer risk. For *BRCA1/2* mutation carriers, risk-reducing salpingo-oophorectomy effectively prevents ovarian carcinoma, reduces the risk of breast carcinoma, and significantly improves all cause mortality (9-11). Secondly, identification of *BRCA1/2* mutations may augment therapeutic options. Inhibitors of poly-ADP ribose polymerase (PARPi), are synthetically lethal to cells deficient in *BRCA1* and *BRCA2* (12, 13), and the potential to use PARPi's therapeutically has provided additional incentive to identify mutation carriers.

Many studies have reported improved survival in *BRCA1/2* mutation carriers with ovarian carcinoma, likely secondary to improved sensitivity to platinum chemotherapy (7, 8, 14-20). However, these studies are often impacted by selection bias. Even prospective population series attempting to test all patients with ovarian carcinoma for *BRCA1/2* mutations have suffered from incomplete enrollment, with nearly 40% of eligible women not enrolled (1-3). The most common reasons for non-enrollment in these studies were death or feeling too sick. Therefore, it is difficult to avoid "survivor" bias (18), as patients who live longer and feel better may be more likely to pursue genetic testing, potentially inflating the survival benefit of having a *BRCA1/2* mutation.

The BROCA test employs targeted gene capture followed by massively parallel sequencing to assess for mutations in multiple cancer susceptibility genes (21). BROCA accurately detects all classes of mutations, including single base-pair substitutions, small insertions and deletions, and large genomic rearrangements. We recently published the results of BROCA testing in a prospective series of 360 ovarian carcinoma patients enrolled at the time of surgery and not selected by family history or age. We identified 17.5% of subjects to have deleterious *BRCA1/2* mutations and an additional 6% to have inherited mutations in other genes (22). Approximately two-thirds of *BRCA1/2* mutations in this study had been previously identified through routine clinical care and commercial testing. Despite the routine recommendation in our practice for referral of all women with invasive ovarian carcinoma to genetic counseling, one-third of *BRCA1/2* mutations had not been previously identified. We wondered if these women differed clinically from those with clinically identified mutations. The goal of this study was to compare clinical characteristics of women with *BRCA1/2* mutations whose mutations were identified through routine clinical care to those who were identified only through the research protocol.

Methods

Patients were retrospectively identified from an IRB-approved gynecologic oncology tissue bank at one institution from the time period of 1/1/1999 – 1/1/2011. Patients within this tissue bank are prospectively enrolled prior to surgery. Criteria for enrollment include having a known or suspected ovarian cancer and understanding the consent form in English. Post-operatively patients are enrolled in the cancer genetics studies prior to hospital discharge. Approximately 95% of eligible patients enroll in our genetics studies. Patients were selected for analysis if they had ovarian, peritoneal, or fallopian tube carcinoma (referred to as ovarian carcinoma for remainder), and had undergone either clinical or research testing for *BRCA1/2* mutations. Clinical genetic testing results were obtained from the medical record. Some patients receive their surgery at our institution but follow up closer to home for chemotherapy. Some of these women were tested locally with our without referral to a genetic counselor. In either case, our practice was to recommend referral to a genetic counselor for all women with invasive non-mucinous ovarian cancer since 2005. We recommend referral regardless of participation in genetics studies, in order to avoid disruption in usual clinical care. However, even currently and despite multiple attempts to alter the counseling and testing system, we find that only the minority of women who meet these criteria do get genetic testing. BROCA testing was performed on banked DNA from patients in the tissue bank who had consented to the genetics studies. Patients were excluded if occult cancer was identified at the time of a risk-reducing salpingo-oophorectomy.

Subjects were divided into three groups: 1) "known": those with known *BRCA1/2* mutations identified through standard commercial testing at any time during their clinical care, 2) "BROCA": those with *BRCA1/2* mutations identified by BROCA testing and not previously identified clinically, and 3) "wildtype": ovarian carcinoma patients without inherited loss of function mutations on BROCA testing including *BRCA1/2* and 19 other tumor suppressor genes (22). Patients with ovarian carcinoma with BROCA-identified germline mutations in other genes (such as in *BRIP1*, *RAD51C*, and *TP53*) were excluded from this analysis. BROCA sequencing was performed on germline DNA as previously reported and all mutations were verified with standard Sanger sequencing (21). *BRCA1/2* mutations were classified as deleterious if they disrupted the open reading frame or caused protein truncation (i.e. frameshift, nonsense, or gene rearrangement), or were a known deleterious missense mutation (as classified on the Breast Cancer Information Core (BIC) Databases, <http://research.nhgri.nih.gov/projects/bic/>, accessed 9/1/2012) (23). Individuals with rare variants of uncertain significance in *BRCA1/2* were excluded.

The medical record was reviewed for clinical information, including genetic testing results and outcomes. A strong family history was defined as any ovarian or breast cancer in the family, excluding a single breast cancer after age 60 in a second degree or greater relative. Optimal debulking was defined as greatest diameter of residual disease after surgical cytoreduction less than 1cm. For histology, carcinomas were labeled undifferentiated when the pathology report indicated "adenocarcinoma," "carcinoma," or "poorly differentiated carcinoma, not otherwise specified." Grade 2 or 3 serous carcinomas were labeled high-grade serous, and grade 1 serous carcinomas were labeled low-grade serous. Overall survival was calculated from the date of diagnosis to the date of death or censored at the last follow-up if still alive. Progression-free survival was calculated from the date of diagnosis to the date of first recurrence or censored at the last follow-up in which the patient was confirmed to be disease free. Analyses of survival, debulking, and use of neoadjuvant chemotherapy were restricted to advanced stage cases (stage II – IV).

Statistical analyses were performed using Prism software (GraphPad, San Diego, CA). Contingency tables were made for comparison of categorical variables, with p values

calculated using Fisher's exact test. The continuous variable of age was assessed with the Mann-Whitney test. Kaplan-Meier survival curves were constructed, with p values calculated using the Log-rank test. Multivariate analyses were performed using JMP software (SAS, Cary, North Carolina). Covariates associated with genetic testing status were assessed with logistic regression. Cox proportional hazards modeling was used to determine predictors of survival in mutation carriers. All p values were two-tailed.

Results

Overall, there were 107 *BRCA1/2* mutation carriers with ovarian carcinoma (including peritoneal, fallopian tube, and ovarian) that were enrolled in the tissue bank. Of these women, 70 were known to have *BRCA1/2* mutations by standard clinical testing (known). An additional 37 *BRCA1/2* mutations were identified through BROCA sequencing in patients that had not undergone commercial testing during their clinical care (BROCA). An additional 291 patients with ovarian carcinoma with negative BROCA testing were used as a control group (wildtype).

Table 1 outlines the clinical characteristics of these three groups. The BROCA mutation carriers were significantly older than the known mutation carriers ($p = 0.003$) and less likely to have a strong family history of breast and ovarian carcinoma ($p < 0.0001$). A personal history of breast carcinoma was present in a similar fraction of BROCA and known mutation carriers. Likewise, there was no difference in rates of optimal debulking, use of neoadjuvant chemotherapy, having a mutation in *BRCA1* vs. *BRCA2*, and the presence of high-grade serous histology. Notably, the BROCA group had a higher proportion of stage IV patients ($p = 0.03$). Using a multivariate model to predict genetic testing status including age at diagnosis, platinum sensitivity, family history, stage, debulking status, and presence of high-grade serous histology; only age (10-year OR 2.85, 95% CI = 1.45 – 6.14, $p = 0.002$) and lack of family history (OR 6.31, 95% CI = 1.99 – 22.51, $p = 0.002$) were independent predictors of not having testing.

The types of mutations in BROCA versus known carriers are listed in Table 2. Mutation class distribution was similar between BROCA and known mutation carriers, except for splice site mutations which were more common in BROCA subjects ($p = 0.048$). The three Ashkenazi Jewish founder mutations in *BRCA1/2* were more common amongst the known mutation carriers ($p = 0.01$).

The median age for all *BRCA1/2* mutation carriers was 53 years (range 33 - 77), significantly younger than wildtype women ($p < 0.0001$). *BRCA1* mutation carriers were younger at diagnosis than *BRCA2* mutation carriers, with a median age of 51 years (range 33 - 77) compared to 56 years respectively (range 43 – 76, $p = 0.01$). Wildtype women were unlikely to have a strong family history or a personal history of breast carcinoma (Table 1). Rates of optimal debulking, use of neoadjuvant chemotherapy, and stage distribution were similar in wildtype women and mutation carriers. Wildtype cases were less likely to have high-grade serous histology than *BRCA1/2* mutation carriers ($p = 0.001$).

Overall survival was significantly worse in BROCA mutation carriers compared with known mutation carriers (median overall survival 45 vs. 93 months, $p < 0.0001$, hazard ratio (HR) 3.47 (95% CI = 1.79 – 6.72), Figure 1A). Median progression free survival was not significantly different between the two groups of mutation carriers (19 vs. 25 months, $p = 0.82$). Using Cox proportional hazards modeling in mutation carriers including the covariates age at diagnosis, platinum resistance, stage, debulking status, presence of high-grade serous histology, and genetic testing status (known vs. BROCA); only platinum resistance (RR 3.06, 95% CI = 1.41 – 6.21, $p = 0.006$) and stage IV disease (RR 2.85, 95%

CI = 1.31 – 6.18, $p = 0.009$) were independent predictors of worse survival. The combined mutation carriers had significantly longer overall survival than wildtype patients (median 69 vs. 44 months, $p = 0.0001$, HR 0.58 (95% CI = 0.43 – 0.77), Figure 1B). Overall survival was similar between BROCA mutation carriers and wildtype women. Therefore, the improved survival in the combined *BRCA1/2* mutation carriers was driven by those with clinically known mutations. While *BRCA2* mutation carriers had a longer median overall survival than *BRCA1* mutation carriers, (82 vs. 66 months), this difference did not achieve statistical significance ($p = 0.58$).

Discussion

Older age, a lack of family history, stage IV disease, and poor survival were associated with decreased clinical identification of genetic risk amongst *BRCA1/2* mutation carriers with ovarian carcinoma. Multivariate analysis revealed that the most significant factors that influenced survival in mutation carriers were platinum sensitivity and stage IV disease. Therefore, the older age and lack of family history in BROCA mutation carriers does not explain their worse survival compared to women with clinically identified mutations. The increase in stage IV disease in the BROCA mutation carriers and its associated impact on survival raises the possibility that poor outcomes from stage IV disease may have been a barrier to clinical genetic testing. Multiple population based studies aimed at determining the prevalence of *BRCA1/2* mutations in women with ovarian carcinoma have reported that the primary reason patients were not enrolled was death or severe illness (1-3). Similarly, sick patients may be less likely to participate in clinical genetic testing. Alternatively, doctors may be less inclined to recommend or pursue genetic testing for very sick patients. In this study, we did not have the clinical information to define why some mutation carriers had not received clinical genetic testing. It is also possible that known mutation carriers were somehow given different treatment that resulted in improved survival, although this explanation seems less likely.

Estimates of how often women with ovarian carcinoma obtain genetic testing are likely to vary by country and by center. Two population-based assessments, from Australia and Canada (where testing is freely available to ovarian carcinoma patients) have shown that 7 – 19% of patients obtain genetic testing, and patients with a family history are more likely to obtain testing (8, 24). In our study, the two factors that were independent predictors of having a mutation detected through standard clinical testing were younger age and the presence of a strong family history. Previous studies have found that *BRCA1/2* mutation carriers have no known family history of breast or ovarian carcinoma 20 – 44% of the time (2, 8, 22, 25). Therefore, referral for genetic testing based on family history alone will miss a substantial number of cases. The National Comprehensive Cancer Network currently lists a personal history of ovarian carcinoma as an indication for genetic testing, with no other requirement for any particular family history (26). The increased uptake of genetic testing amongst women with strong family histories likely results from both patient and provider biases.

We found that *BRCA1/2* mutation carriers had superior overall survival when compared with wildtype patients. This finding has already been well established in multiple previous publications (7, 8, 14-20). What is interesting is that the *BRCA1/2* mutation carriers with clinically undetected mutations (BROCA) had a survival curve similar to the wildtype patients, indicating that the clinically identified mutation carriers are those with the improved survival. Therefore, including only routinely identified *BRCA1/2* mutation carriers will introduce significant survival bias into analyses of outcomes amongst women with *BRCA1/2* associated ovarian carcinoma. Consistent with other published data, we identified a similar rate of optimal debulking between mutation carriers and wildtype (17), and a

higher percentage of high-grade serous histology in mutation carriers than wildtype (5). We do not recommend using high-grade serous histology as criteria for genetic testing as all other histologic subtypes (with the exception of mucinous) have been associated with *BRCA1/2* mutations in this series.

Detection of *BRCA1/2* mutations is clinically important for women with ovarian carcinoma and their families. Poly-ADP ribose polymerase (PARP) inhibitors have recently been developed, which selectively kill cells deficient in *BRCA1* or *BRCA2* (12, 13). PARP inhibitors have demonstrated good response rates in patients with *BRCA1/2* cancers (27, 28) and are currently being studied in multiple clinical trials. PARP inhibitors will likely be an important therapeutic modality for women with *BRCA1/2* associated ovarian carcinoma and provide incentive for identifying mutations in newly diagnosed patients. Additionally, identifying the *BRCA1/2* mutation facilitates clarification of risk to family members, allowing targeted prevention in mutation carriers prior to disease development and avoidance of unnecessary intervention in those not at risk.

There are many barriers that prevent women with ovarian carcinoma from obtaining genetic testing. This study did not assess cost, access to genetic counseling, lack of physician referral, or patient refusal of referral or testing. However, we demonstrated that *BRCA1/2* mutation carriers who are older, lack a family history, have stage IV disease, or have poor survival are less likely to have their mutations clinically detected. In order to avoid missing a substantial number of mutations, women should be tested soon after diagnosis without regard to age or family history. Next generation sequencing techniques (such as the BROCA test) have the ability to accurately test for mutations in multiple genes simultaneously, which will facilitate the identification of an increased fraction of women with hereditary ovarian carcinoma.

Acknowledgments

The authors would like to thank Dr. Mary Claire King and Dr. Barbara Goff for their guidance and support.

Financial support: This work was supported by National Institutes of Health Grants RO1CA131965, RO1CA157744, and P50CA083636, the Breast Cancer Research Foundation, Susan G. Komen for the Cure, and Department of Defense Ovarian Cancer Research Program OC093285.

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Research Highlights

- Older age, lack of family history, and poor survival are associated with decreased clinical identification of *BRCA1/2* mutations.
- Testing should be initiated at time of diagnosis to maximize identification and minimize survival bias.

Figure 1. Ovarian carcinoma overall survival by mutation status

A. Overall survival comparing BROCA to known *BRCA1/2* mutation carriers. BROCA mutation carriers had significantly worse survival (median 45 vs. 93 months, $p < 0.0001$, HR 3.47 (95% CI 1.79 – 6.72)). Wildtype patients are included for reference; BROCA and wildtype were not significantly different. B. Overall survival comparing all *BRCA1/2* mutation carriers (BROCA and known combined) with wildtype patients. *BRCA1/2* mutation carriers had significantly improved survival (median 69 vs. 44 months, $p = 0.0001$, HR 0.58 (95% CI 0.43 – 0.77)).

Table 1
Clinical characteristics of BRCA and known BRCA1/2 mutation carriers, and wildtype

	BRCA <i>BRCA1/2</i>	Known <i>BRCA1/2</i>	p – value (BRCA vs. Known)	Wildtype	p – value (WT vs. all <i>BRCA1/2</i>)
N	36	71		291	
Median Age (Range)	58 (41-77)	51 (33 – 76)	p = 0.003	62 (29-91)	p < 0.0001
Strong Family History	15/37 (40.5%)	58/70 (82.9%)	p < 0.0001	46/291 (15.8%)	p < 0.0001
History of Breast Carcinoma	7/37 (18.9%)	22/70 (31.4%)	p = 0.25	11/291 (3.8%)	p < 0.0001
Optimal Debulking	24/35 (68.6%)	49/60 (81.7%)	p = 0.21	209/276 (75.7%)	p = 0.89
Neoadjuvant Chemotherapy	9/37 (24.3%)	10/66 (15.2%)	p = 0.29	55/276 (19.9%)	p = 0.88
% <i>BRCA1</i>	25/37 (67.6%)	54/70 (77.1%)	p = 0.36	NA	NA
Histology:					
HG Serous	30 (81.1%)	57 (81.4%)	p = 1.0 (% HG serous)	188 (64.6%)	p = 0.001 (% HG serous)
Undifferentiated	5 (13.5%)	6 (8.6%)		53 (18.2%)	
LG Serous	1 (2.7%)	0		5 (1.7%)	
Endometrioid	0	6 (8.6%)		19 (6.5%)	
Clear Cell	1 (2.7%)	0		12 (4.1%)	
Mucinous	0	0		4 (1.4%)	
Carcinosarcoma	0	1 (1.4%)		10 (3.4%)	
FIGO Stage:					
Stage I	0 (0%)	4 (5.7%)		15 (5.2%)	
Stage II	1 (2.8%)	4 (5.7%)		22 (7.6%)	
Stage III	23 (62.2%)	51 (72.9%)		198 (68.0%)	
Stage IV	13 (35.1%)	11 (15.7%)	p = 0.03(% stage IV)	56 (19.2%)	p = 0.78(% stage IV)

Abbreviations: WT = wildtype, HG = high-grade, LG = low-grade

Table 2Mutations in BROCA vs. known *BRCA1/2* mutation carriers.

Mutation Class	BROCA <i>BRCA1/2</i> N=37	Known <i>BRCA1/2</i> N=70	p – value
Frameshift	20 (54%)	47 (67.1%)	NS
Nonsense	9 (24.3%)	14 (20.0%)	NS
Missense	2 (5.4%)	5 (7.1%)	NS
Rearrangement	2 (5.4%)	3 (4.3%)	NS
Splice Site	4 (10.8%)	1 (1.4%)	p=0.048
* Ashkenazi Jewish Founder Mutation	2/37 (5.4%)	18/70 (25.7%)	p = 0.01

* Ashkenazi Jewish Founder mutations: *BRCA1*.187delAG, *BRCA1*.5385insC, and *BRCA2*.6174delT

NS = nonsignificant