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## Germline mutation in *BRCA1* or *BRCA2* and ten-year survival for women diagnosed with epithelial ovarian cancer

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### Abstract

**Purpose**—To analyse the effect of germline mutations in *BRCA1* and *BRCA2* on mortality in ovarian cancer patients up to ten years after diagnosis.

**Experimental Design**—We used unpublished survival time data for 2,242 patients from two case-control studies and extended survival-time data for 4,314 patients from previously reported studies. All participants had been screened for deleterious germline mutations in *BRCA1* and *BRCA2*. Survival time was analysed for the combined data using Cox proportional hazard models with *BRCA1* and *BRCA2* as time-varying covariates. Competing risks were analysed using Fine and Gray model.

**Results**—The combined 10-year overall survival was 30% (95% CI, 28%-31%) for non-carriers, 25% (95% CI, 22%-28%) for *BRCA1* carriers, and 35% (95% CI, 30%-41%) for *BRCA2* carriers. The hazard ratio for *BRCA1* was 0.53 at time zero and increased over time becoming greater than one at 4.8 years. For *BRCA2*, the hazard ratio was 0.42 at time zero and increased over time (predicted to become greater than one at 10.5 years). The results were similar when restricted to 3,202 patients with high-grade serous tumors, and to ovarian cancer specific mortality.

**Conclusions**—*BRCA1/2* mutations are associated with better short-term survival, but this advantage decreases over time and, in *BRCA1* carriers is eventually reversed. This may have important implications for therapy of both primary and relapsed disease and for analysis of long-term survival in clinical trials of new agents, particularly those that are effective in *BRCA1/2* mutation carriers.

### Keywords

Ovarian cancer; Epithelial ovarian cancer; *BRCA1* gene; *BRCA2* gene; Survival

### Introduction

Epithelial ovarian cancer (EOC) is the most fatal gynecological malignancy, resulting in ~140 000 deaths worldwide per year (1). EOC is a heterogeneous disease with multiple histopathological sub-types that is usually treated using a combination of cytoreductive surgery and platinum-based chemotherapy (2). However, women often present with

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advanced-stage disease and the prognosis is generally poor. Clinical management of the disease might be improved by a more personalized approach to treatment based on likely treatment response. Germline mutations in *BRCA1* and *BRCA2* are associated with a high risk of EOC, predominantly of the high-grade serous sub-type (HGSOC). Mutations in these genes account for 5 to 15 per cent of all cases of EOC (3-6). There is substantial evidence that HGSOC patients with *BRCA1* or *BRCA2* germline mutations have better short-term survival than non-carriers (6, 7), but recent studies suggested that this survival advantage did not persist after five years (8, 9).

We have recently sequenced *BRCA1* and *BRCA2* in two large EOC case series in order to estimate the contribution of these genes to EOC in the general population (10). Long-term outcome data were also available for these cases. In the study reported by Bolton et al (7), cause-specific mortality data were not available, those analyses had been restricted to the first five years after diagnosis when it was assumed that most deaths would be due to ovarian cancer. Alsop et al considered disease-specific mortality with 5.3 years median follow-up (6). However, long-term all-cause mortality data are also available for both studies. The aim of the current analysis was to determine the effect of *BRCA1* and *BRCA2* mutation status on long-term survival in women with EOC.

## Patients and Methods

### Patients

We used survival time data for 6,556 EOC cases from 27 studies. Two case-control studies, the population-based SEARCH study (n=1,419) and the clinic-based Mayo clinic study (n=823), were screened for deleterious mutations in *BRCA1* and *BRCA2* using multiplexed 48.48 Fluidigm access arrays for targeted sequence library preparation followed by sequencing on an Illumina HiScan sequencer (10). In addition, we used extended survival time data for 3,325 cases previously reported by Bolton et al (7), and for 989 cases from the Australian Ovarian Cancer Study (AOCS) (6). Some cases from SEARCH and the Mayo-clinic study were included in the Bolton et al analysis. These duplicates excluded for this analysis. Number of individuals by *BRCA* status and references describing each study design are given in Supplementary Table S1.

We considered protein-truncating insertion/deletion variants, consensus splice-site variants and missense variants with reported damaging effect on protein function to be deleterious. For the purpose of our analysis, *BRCA1* and *BRCA2* mutation status were recorded simply as mutation-positive or negative, with no distinction between different mutation types by location or functional effect.

### Statistical analysis

We used standard Cox regression with a primary end point of death from all cause for the survival analysis. Survival time was from the date of diagnosis until the date of death. For the 3,075 cases from 12 studies with cause of death available, we used Fine and Gray competing risks regressions to predict 10-year probability of death from ovarian cancer - sub distribution hazard ratios (SHR) for ovarian cancer. The Fine and Gray model is a

multivariable time-to-event model, which accounts for the fact that individuals can only have one competing event. The model also accounts for censoring among those who do not have an event during follow-up (11). Participants were recruited at a variable time after diagnosis, which was allowed for in the analyses by treating time at risk from the date of recruitment (left truncation). This results in an unbiased estimate of the hazard ratio provided the proportional hazard assumption is valid (12). In preliminary analyses tests of the proportional hazards assumption using Schoenfeld residuals showed that the assumption was seriously violated for both *BRCA1* and *BRCA2*, which would be expected if the hazard ratio changes over time as suggested by McLaughlin et al (8). We therefore modelled the hazard ratios for *BRCA1* and *BRCA2* by treating them as time-varying covariates such that the log hazard ratio varies linearly with time. The hazard ratio at time *t* is then given by

$$HR(t) = \exp(\beta x + \delta xt)$$

where *x* is the predictor variable (*BRCA1* or *BRCA2* status),  $\beta$  is the  $\beta$ -coefficient and  $\delta$  is the time varying coefficient. Under the proportional hazards assumption  $\delta$  equals zero.

All analyses were stratified by year of diagnosis (before 1990; 1990-1995; 1996-1999; 2000 and after) and study. The covariates in multi-variable models were: age at diagnosis (measured in years), clinical stage (localized (IA, IB), regional (IC-II) and distant (III/IV)), histopathological grade (low=Grade 1/well differentiated or high=Grade 2/Grade 3/poorly differentiated) and morphological sub-type (serous or non-serous).

There was missing data for a substantial proportion of cases for stage (12 per cent) and grade (17 per cent). Multiple imputation has been shown to be the method for the handling of missing data that is least likely to be biased across a wide range of assumptions. We therefore imputed twenty complete data sets for each study using multivariate imputation by chained equations (13). The imputation model included *BRCA1* and *BRCA2* mutation status, year of diagnosis, age at diagnosis, morphological sub-type, outcome, time of follow up and study. Each imputed data set was analysed separately and the parameter estimates were combined according to ‘‘Rubin’s rules’’ (14).

Differences in time elapsed from diagnosis to entry in study, follow-up time, year of diagnosis, proportion of deaths from ovarian cancer, tumor histology, grade, stage, and age at diagnosis were tested using *t* and  $X^2$  tests. Statistical analysis was conducted using STATA/SE version 13 (StataCorp).

## Results

The characteristics of the patients are shown in Table 1. In the SEARCH case series there were 41 *BRCA1* mutation carriers, 59 *BRCA2* mutation carriers and 1,319 cases without a mutation in either gene. In the Mayo clinic case series there were 38 *BRCA1* mutation carriers, 27 *BRCA2* mutation carriers and 758 cases without a mutation in either gene. In the AOCs case series there were 89 *BRCA1* mutation carriers, 54 *BRCA2* mutation carriers and 846 cases without a mutation in either gene (6). There were 890 *BRCA1* carriers, 298

*BRCA2* carriers and 2137 non-carriers from the study previously published by Bolton et al (7).

The crude 5-year overall survival was 42% (95% CI, 41%-44%) for non-carriers, 45% (95% CI, 41%-48%) for *BRCA1* carriers, and 54% (95% CI, 48%-59%) for *BRCA2* carriers. The 10-year overall survival was 30% (95% CI, 28%-31%) for non-carriers, 25% (95% CI, 22%-28%) for *BRCA1* carriers, and 35% (95% CI, 30%-41%) for *BRCA2* carriers (Fig 1). Based on the multi-variable analysis of the imputed data, the hazard ratio for *BRCA1* at time zero ( $t_0$ ) was 0.53 (0.43 – 0.66,  $P < 0.001$ ) which increased significantly with time (coefficient for time-by-covariate interaction = 1.14, 95% CI 1.08 – 1.20,  $P < 0.001$ ) (table 2). The hazard ratio for *BRCA1* positivity at time  $t$  is thus given by the formula

$$HR(t) = \exp(-0.63 + 0.13t)$$

This means that the HR for *BRCA1* is less than one from  $t=0$  to  $t=4.8$  years and is greater than one after  $t=4.8$  years.

The multi-variable adjusted hazard ratio for *BRCA2* at  $t_0$  was 0.42 (0.30 – 0.59,  $P < 0.001$ ) and this increased significantly with time (coefficient for time-by-covariate interaction = 1.09, 95% CI 1.01 – 1.19,  $P = 0.048$ ) (Table 2). The hazard ratio for *BRCA2* positivity at time  $t$  is thus given by the formula

$$HR(t) = \exp(-0.87 + 0.08t)$$

This means that the HR for *BRCA2* is less than one from  $t=0$  to  $t=10.5$  years and is greater than one after  $t=10.5$  years. The hazard ratios for the complete-case analysis were similar to those for the analysis of the imputed data but, as expected, the standard errors of the hazard ratio estimates were larger and the findings less significant (Supplementary Table S2).

We also analysed a subset of 3,075 cases (256 *BRCA1* mutation carriers, 168 *BRCA2* mutation carriers and 2,651 cases without a mutation in either gene) for whom cause of death was available. There were 147 (5.5%) non-ovarian cancer deaths among non-carriers, 10 (3.9%) among *BRCA1* carriers and 7 (4.2%) among *BRCA2* carriers. Based on the competing risks regressions analysis of the imputed data, the SHRs at  $t_0$  were 0.42 (0.30 – 0.60,  $P < 0.001$ ) for *BRCA1* carriers and 0.34 (0.22 – 0.54,  $P < 0.001$ ) for *BRCA2* carriers. The coefficients for time-by-covariate interaction were 1.19 (1.10 – 1.29,  $P < 0.001$ ) for *BRCA1* carriers and 1.16 (1.05 – 1.28,  $P = 0.005$ ) for *BRCA2* carriers. The SHRs were greater than 1 after 4.9 years for *BRCA1* and after 7.3 years for *BRCA2* (Supplementary Tables S3 and S4).

Ovarian cancer in *BRCA1* and *BRCA2* carriers is usually the high-grade serous subtype. We therefore repeated these analyses for all-cause mortality restricting the data to the subset of 3,202 HGSOC cases (470 *BRCA1* mutation carriers, 216 *BRCA2* mutation carriers and 2,516 cases without a mutation in either gene). Based on the multi-variable analysis of the imputed data, the HRs at  $t_0$  were 0.51 (0.38 – 0.68,  $P < 0.001$ ) for *BRCA1* carriers and 0.34 (0.22 – 0.54,  $P < 0.001$ ) for *BRCA2* carriers. The coefficients for time-by-covariate interaction were 1.15 (1.07 – 1.23,  $P < 0.001$ ) for *BRCA1* carriers and 1.12 (1.002 – 1.24,  $P = 0.045$ ) for *BRCA2*

carriers. The HRs were greater than 1 after 4.9 years for *BRCA1* and after 9.7 years for *BRCA2* carriers (Supplementary Tables S5 and S6).

## Discussion

Consistent with previously published studies (8, 15-17) we found that patients with epithelial ovarian cancer carrying *BRCA1* or *BRCA2* mutations have better short-term survival (5 years) than non-carriers. This survival advantage was lost over time and after approximately five years *BRCA1* carriers had a higher risk of dying than non-carriers. Also consistent with the generally better short-term survival of *BRCA2* carriers compared with germline *BRCA1* mutation carriers, a survival advantage persisted longer in *BRCA2* patients and did not cross-over with non-carriers until approximately nine years after diagnosis.

The large sample size of the current study - including previously unpublished data on 165 *BRCA1* or *BRCA2* mutation carriers and 2,077 non-carriers in addition to data on 4,714 cases that were previously published as part of an analysis of short-term survival is a major strength of the current analysis. The large sample size allowed us to analyze data from the subset of patients with high-grade serous cancer, thereby excluding low-grade cases that can have more indolent disease and are less likely to carry mutations in *BRCA1* or *BRCA2*. Hence it is unlikely that contamination by low-grade tumours, which may have been simply cured surgically, contributed to the favourable long-term survival of non-carriers.

We have no information on recurrence for a large number of the cases and cause of death was not available for 15 studies (3,481 cases); consequently, primary analyses were based on all-cause mortality. The proportion of deaths from causes other than ovarian cancer was small in the studies with data on cause-specific mortality, as has been reported in other ovarian cancer case series (18). It is likely that the majority of deaths occurring in the first five years after diagnosis were due to ovarian cancer and so any misclassification will have been minimal. The comparison of all-cause mortality by *BRCA1* and *BRCA2* carrier status over the long term may not reflect differences in ovarian cancer specific mortality if non-ovarian cancer mortality also differs between carriers and non-carriers. This is likely to be true as carriers are also at increased risk of other cancers. However, over the longer term, competing causes of mortality become more important. We therefore performed an analysis restricting the data to those cases with information on cause-specific mortality using an analytic approach that allows for competing risks. The findings were broadly similar to the results for all-cause mortality suggesting that differences in non-ovarian cancer mortality do not account for the time dependent effect for *BRCA1* and *BRCA2* carriers.

The primary studies are heterogeneous in design and patient management is likely to have varied substantially across studies. This heterogeneity is a strength as it suggests that our findings are robust and generalizable. However, lack of detailed data on treatment limits our ability to investigate interactions between mutation status and specific treatments. In particular, investigation of hypotheses regarding revertant mutations or intra-tumoral heterogeneity need detailed progression-free survival and response data. These data may be available in the future from retrospective analysis of large multicentre trials such as ICON7 and ICON8.

Exclusion of important prognostic factors from a Cox model may result in other variables behaving as time varying covariates (19). Our findings may therefore be due to the fact that we did not include residual disease as a covariate in the prognostic models (these data were not available in our case series). However, simulations excluding other important prognostic variables, such as clinical stage, had little impact on the magnitude of the coefficients for the time dependent effects (data not shown), suggesting that exclusion of other covariates is unlikely to be an explanation for our findings.

The reasons why *BRCA1/2* carriers have only a short-term survival advantage are not clear. However, while 10 years survival may reflect the cure from their disease, 5-year survival would allow for a proportion of patients who are still alive with incurable disease. *BRCA1* and *BRCA2* are important in double strand break DNA repair by homologous recombination (20, 21) and cell lines deficient in *BRCA1* and *BRCA2* function are more sensitive to platinum (22, 23). Furthermore, presence of germline and somatic homologous recombination mutations is predictive of primary platinum sensitivity in women with EOC (24). Carrier status may initially segregate those patients with platinum sensitivity from high-grade serous cancer patients whose tumors lack HR defects, such as those with *CCNE1* amplification (25), who are frequently resistant to primary therapy and have poor outcomes (26).

Intragenic reversion of germline alleles that restore *BRCA1* and *BRCA2* function in tumor cell lines (27) and in recurrent ovarian carcinomas (28) has been observed and it is possible that this is associated with a time-dependent loss of the survival advantage associated with germline mutation. A minority of HGSC patients achieve long-term remissions following optimal debulking surgery and chemotherapy, where presumably adjuvant treatment is able to successfully eradicate any cancer repopulating cells remaining after surgery. Our findings may reflect differences between carriers and non-carriers in the abundance of cancer stem cells or the ability of those cells to be ablated by adjuvant treatment or host immunological factors. Indeed, expansion of the breast luminal progenitor population is observed in *BRCA1* mutation carriers (29), suggesting that partial loss of HR function can influence the stem cell kinetics. Intra-tumour genetic heterogeneity at the time of primary treatment may comprise an alternative mechanism for acquired platinum resistance.

Despite the advances in the understanding of the genetics and biology of ovarian cancer during the past ten years, the clinical management of the disease remains challenging. Our findings confirm that germline genotype is an important predictor of response to treatment in both the short- and long-term and emphasises the need to identify novel approaches to the management of the disease that target the underlying biology.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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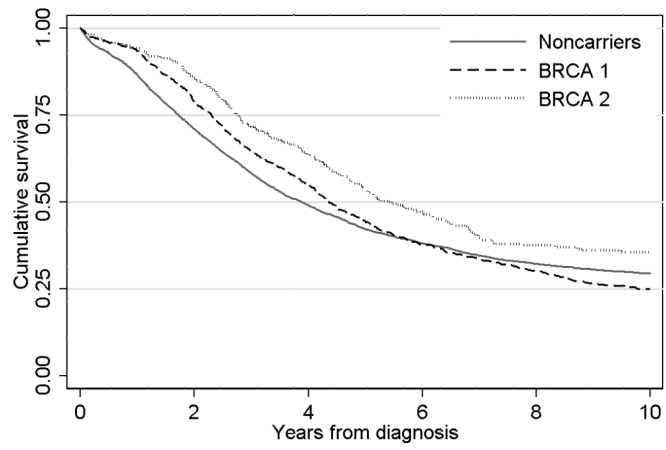
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### Translational Relevance

Previous studies show consistent association between BRCA1 or BRCA2 germline mutations and improved 5-year survival in ovarian cancer. However, recent studies suggested that this survival advantage did not persist after five years.

This is a large and comprehensive study, which has investigated the role of *BRCA1/2* status on long term survival of ovarian cancer patients. We confirmed the hazard ratios for death associated with *BRCA1/2* germline mutations is lower than 1.0 at diagnosis, however, it increases over time. These findings were independent of other clinical prognostic factors including histological subtype. These results are of fundamental importance for counselling patients about their prognosis and in interpreting results of clinical trials involving *BRCA1/2* carriers.



	0	2	4	6	8	10
Number at risk						
Noncarriers	1924	2914	2295	1657	1120	812
Carriers						
BRCA1	404	676	499	323	198	118
BRCA2	162	278	239	152	86	58

**Figure 1. Kaplan-Meier Estimates of Cumulative Survival According to BRCA1/2 Mutation Status**

**Table 1**  
**Characteristics of Study Participants**

Characteristic	Noncarriers (5,060)		<i>BRCA1</i> mutation (1,058)		<i>BRCA2</i> mutation (438)	
	N	%	N	%	N	%
Histology						
Serous	3,052	65	553	73	254	77
Mucinous	271	6	5	0.7	1	0.3
Endometrioid	587	12	91	12	27	8
Clear cell	306	6	14	2	7	2
Mixed cell	150	3	8	1	7	2
Other	345	7	82	11	32	10
Unknown	349	-	305	-	110	-
Grade						
Low	491	11	19	2	13	4
High	3,778	88	801	98	331	96
Unknown	791	-	238	-	94	-
Stage						
Localized	967	21	97	12	29	8
Regional	818	18	101	12	55	15
Distant	2808	61	622	76	274	76
Unknown	467	-	238	-	80	-
Age at EOC diagnosis, mean (SD), year	59(11)		52 (10)		59 (10)	

The cases with unknown histology, grade and stage were not included in the calculation of proportions.

**Table 2**  
**Ten years estimated hazard ratios (HR) for death in patients with ovarian cancer**

Variable (Reference)	Conventional Cox model			Cox model with time-varying-effect		
	HR	95% CI	p-value	HR	95% CI	p-value
Age (per year)	1.02	1.02-1.02	<0.001	1.02	1.02-1.02	<0.001
Grade (ref: low)	1.66	1.40-1.96	<0.001	1.64	1.38-1.94	<0.001
Regional (ref: localized)	2.89	2.41-3.47	<0.001	2.87	2.40-2.44	<0.001
Distant (ref: localized)	6.64	5.58-7.90	<0.001	6.59	5.54-7.84	<0.001
Serous (ref: non-serous)	1.08	0.99-1.17	0.10	1.07	0.98-1.17	0.13
BRCA1 (ref: non carriers)	0.83	0.74-0.93	0.002	0.53	0.43-0.66	<0.001
BRCA2 (ref: non carriers)	0.55	0.47-0.65	<0.001	0.42	0.30-0.59	<0.001
BRCA1*_t				1.14	1.08-1.20	<0.001
BRCA2*_t				1.09	1.01-1.18	0.048

\*\_t: time-varying Hazard Ratio