Title

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Journal

Clinical Cancer Research, 20(3)

ISSN

1078-0432

Authors

Pennington, Kathryn P
Walsh, Tom
Harrell, Maria I
et al.

Publication Date

2014-02-01

DOI

10.1158/1078-0432.ccr-13-2287

Peer reviewed

Published in final edited form as:
Clin Cancer Res. 2014 February 1; 20(3): 764–775. doi:10.1158/1078-0432.CCR-13-2287.

Germline and Somatic Mutations in Homologous Recombination Genes Predict Platinum Response and Survival in Ovarian, Fallopian Tube, and Peritoneal Carcinomas

Kathryn P. Pennington[1], **Tom Walsh**[2], **Maria I. Harrell**[1], **Ming K. Lee**[2], **Christopher C. Pennil**[1], **Mara H. Rendi**[3], **Anne Thornton**[2], **Barbara M. Norquist**[1], **Silvia Casadei**[2], **Alexander S. Nord**[2], **Kathy J. Agnew**[1], **Colin C. Pritchard**[4], **Sheena Scroggins**[4], **Rochelle L. Garcia**[3], **Mary-Claire King**[2], and **Elizabeth M. Swisher**[1,2]

[1]Division of Gynecologic Oncology, University of Washington Medical Center, Seattle, WA

[2]Division of Medical Genetics, University of Washington Medical Center, Seattle, WA

[3]Department of Pathology, University of Washington Medical Center, Seattle, WA

[4]Department of Laboratory Medicine, University of Washington Medical Center, Seattle, WA

Abstract

Purpose—Hallmarks of germline *BRCA1/2*-associated ovarian carcinomas include chemosensitivity and improved survival. The therapeutic impact of somatic *BRCA1/2* mutations and mutations in other homologous recombination (HR) DNA repair genes is uncertain.

Experimental Design—Using targeted capture and massively parallel genomic sequencing, we assessed 390 ovarian carcinomas for germline and somatic loss-of-function mutations in 30 genes, including *BRCA1*, *BRCA2*, and 11 other genes in the HR pathway.

Results—31% of ovarian carcinomas had a deleterious germline (24%) and/or somatic (9%) mutation in one or more of the 13 HR genes: *BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*. Non-serous ovarian carcinomas had similar rates of HR mutations to serous carcinomas (28% vs. 31%, p=0.6), including clear cell, endometrioid, and carcinosarcoma. The presence of germline and somatic HR mutations was highly predictive of primary platinum sensitivity (p=0.0002) and improved overall survival (p=0.0006), with median overall survival 66 months in germline HR mutation carriers, 59 months in cases with a somatic HR mutation, and 41 months for cases without an HR mutation.

Conclusions—Germline or somatic mutations in HR genes are present in almost one-third of ovarian carcinomas, including both serous and non-serous histologies. Somatic *BRCA1/2* mutations and mutations in other HR genes have a similar positive impact on overall survival and platinum responsiveness as germline *BRCA1/2* mutations. The similar rate of HR mutations in non-serous carcinomas supports their inclusion in PARP inhibitor clinical trials.

Keywords

Ovarian carcinoma; next-generation sequencing; homologous recombination; somatic mutations; survival

Corresponding author: Elizabeth M. Swisher MD, University of Washington Medical Center, Department of Obstetrics and Gynecology, Box 356460, Seattle, Washington 98195-6460, Phone: 206-543-3669, Fax: 206-543-3915, swishere@u.washington.edu.

No potential conflicts of interest were disclosed.

INTRODUCTION

Inherited mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) account for the majority of familial ovarian carcinoma (1). *BRCA1/2*, along with other genes in the Fanconi anemia (FA) pathway, play key roles in homologous recombination (HR), the main mechanism that repairs double-strand DNA breaks. Other FA-BRCA genes have also been implicated in genetic susceptibility to ovarian carcinoma including *BRIP1*, *RAD51C*, and *RAD51D* (1–4). Hallmarks of *BRCA1/2*-associated ovarian carcinomas include sensitivity to platinum chemotherapy, improved overall survival (5–10), and sensitivity to poly(ADP-ribose) polymerase inhibitors (PARPi) (11, 12). In addition to *BRCA1/2*, mutations in other FA-BRCA genes may impact HR function and increase sensitivity to DNA damaging agents. *In vitro* studies demonstrate that deficiency in other HR proteins such as ATM, CHEK1, CHEK2, NBN, and RAD51D also confer sensitivity to PARPi (2, 13). PARPi are active agents in ovarian carcinomas from women with germline *BRCA1/2* mutations, but also in a subset of "sporadic" recurrent platinum-sensitive ovarian carcinomas (12). Indeed, the Cancer Genome Atlas (TCGA) reported HR defects in approximately 50% of high-grade serous ovarian carcinomas (14). The availability of PARPi as therapeutic agents adds incentive to better characterize this subset of ovarian carcinoma.

We hypothesize that somatic and germline mutations in a variety of FA-BRCA genes could identify those subjects with "sporadic" ovarian carcinoma whose cancers are sensitive to PARPi, and that these cases will have increased sensitivity to platinum chemotherapy and prolonged survival, as do individuals with germline *BRCA1/2* mutations. We therefore sought to determine the rate of germline and somatic mutations in 13 HR genes in a series of women with ovarian, fallopian tube, and peritoneal carcinoma, and to correlate the presence of these mutations with response to platinum-based chemotherapy and overall survival.

RESULTS

367 individuals and 390 carcinomas were included in the study: 310 individuals with primary carcinoma, 34 with recurrent carcinoma, and 23 with a paired primary and recurrent carcinoma. Of the 367 subjects, 304 had ovarian carcinoma, 24 had fallopian tube carcinoma, 32 had peritoneal carcinoma, and 7 had synchronous ovarian and endometrial carcinomas. Table 1 provides characteristics of cases included in the study. Most cases were advanced-stage (83%), of either serous histology or poorly-differentiated adenocarcinoma (83%), and were optimally cytoreduced (66%, to <1cm maximal residual tumor diameter) at the time of primary surgery. All primary carcinomas received platinum-based chemotherapy, with the exception of five stage I carcinomas. Targeted capture by BROCA baits and genomic sequencing yielded median 289-fold coverage; the percent of targeted bases at >10x and >50x depth was 99% and 93%, respectively.

Overall HR mutation rate

Eighty-seven subjects (24%) had a germline HR mutation, and 32 subjects (9%) had a somatic HR mutation (Supplementary Table 1). Four subjects (1.1%) had both a germline and somatic HR mutation (Supplementary Table 2). Thus, the total proportion of subjects with at least one loss-of-function germline or somatic HR mutation was 31% (115/367) (Figure 1A). Of the 123 germline and somatic HR mutations, 68 (55%) occurred in *BRCA1*, 23 (19%) in *BRCA2*, and 32 (26%) in 11 other HR genes: *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*. Of the four cases with both germline and somatic HR mutations, one case had both a germline (816delGT) and somatic *BRCA1* mutation (del exon 1–2). In this case, the somatic mutation may represent the "second hit" inactivating the wildtype *BRCA1* allele. In the remaining three cases, the somatic mutation represented only a smaller fraction (20–35%) of the DNA sequences in the

neoplasm. Presumably, in these cases the germline mutation was the driver and the somatic mutation was incidental.

Germline mutations

Ninety-four loss-of-function germline mutations were identified in the 367 subjects in 15 different genes. Eighty-seven subjects (24%) had 88 germline mutations in HR genes, while 6 (1.6%) subjects had mutations in non-HR genes including 3 in *TP53*, 1 in *MSH2*, 1 in *BUB1B*, and *1* in *MSH6*. The 88 germline loss-of-function mutations in 11 HR genes included 49 (56%) in *BRCA1*, 17 (19%) in *BRCA2*, and 22 (25%) in other HR genes: 2 (2%) in *BARD1*, 4 (4.5%) in *BRIP1*, 1 (1%) in *CHEK1*, 3 (3%) in *CHEK2*, 2 (2%) in *FAM175A*, 1 (1%) in *NBN*, 2 (2%) in *PALB2*, 3 (3%) in *RAD51C*, and 4 (4.5%) in *RAD51D* (Figure 1B, Supplementary Table 1). One subject had germline mutations in both *MSH6* and *BRIP1*, as previously reported (1). One subject had a germline nonsense mutation in *RAD50* (p.Y625X). However, several other nonsense and frameshift mutations in *RAD50* are relatively common in the North American population, as reported on the exome variant server (http://evs.gs.washington.edu/EVS/, April 2013). Therefore, the clinical significance of inactivation of one *RAD50* allele is questionable and heterozygous *RAD50* mutations were not included in the HR-deficient category.

Somatic mutations

Thirty-two of 367 subjects (8.7%) had a total of 35 somatic loss-of-function mutations. The 35 mutations occurred in 7 HR genes: 19 (54%) in *BRCA1*, 6 (17%) in *BRCA2*, 3 (9% %) in *ATM*, 2 (6 %) in *BRIP1*, 3 (9%) in *CHEK2*, 1 (3%) in *MRE11A*, and 1 (3%) in *RAD51C* (Figure 1C). Supplementary Table 1 details all deleterious germline mutations, somatic HR mutations, somatic *PTEN* mutations, and accompanying case characteristics. One subject had a *RAD50* gene rearrangement, which was excluded. 290 cases (79%) had a deleterious somatic *TP53* mutation. *TP53* mutations in serous carcinomas were limited to grade 2–3 carcinomas. However, *TP53* mutations were also observed in other histologies, including 3/19 clear cell, 11/26 endometrioid (including one grade 1 carcinoma), 10/12 carcinosarcoma, 1/1 malignant Brenner's, and 1/2 mixed histologies.

The role of *PTEN* in HR deficiency is controversial, and we did not classify *PTEN* mutations as HR-deficient. Twenty-two cases (6%) had somatic *PTEN* loss-of-function mutations, including four which had an accompanying HR germline mutation and two which had an accompanying somatic HR mutation.

Non-serous histology

Sixty-one cases (17%) were of non-serous histology, including 19 clear cell, 26 endometrioid, 12 carcinosarcoma, 2 mixed with predominant endometrioid histology, 1 mucinous, and 1 malignant Brenner's carcinoma. Seventeen of 61 (28%) non-serous cases had a deleterious germline or somatic HR mutation (Table 2). Similarly, 80 of 258 (31%) serous cases had a germline or somatic HR mutation (p=0.63) (Figure 2A). Loss-of-function HR mutations were identified in almost every type of non-serous histology tested, including 5/19 (26%) clear cell, 7/26 (27%) endometrioid, 4/12 (33%) carcinosarcoma, and 1/1 (100%) malignant Brenner's carcinoma (Figure 2A). No HR mutations were identified in the one mucinous or two mixed histology carcinomas. Interestingly, 2 of 6 (33%) low-grade endometrioid carcinomas had HR mutations (Table 2). In the 9 subjects with low-grade serous carcinoma included in the study, one (11%) had an HR mutation. While there was a predominance of *BRCA1*/2 mutations in serous cases, non-serous histologies had a wider distribution of mutations in genes other than *BRCA1/2* (Figure 2B). In the non-serous carcinomas with germline or somatic HR mutations (collectively), 56% (10 of 18) of

mutations were in genes other than *BRCA1/2*. In contrast, only 21% (18 of 85) of HR mutations in serous carcinomas had mutations in other HR genes (p=0.005).

Primary platinum response

243 of 333 (73%) subjects with primary carcinoma had adequate clinical information available to define primary platinum response, with platinum sensitivity defined as maintenance of complete response ≥6 months post completion of platinum therapy. The presence of a germline or somatic mutation in an HR gene was strongly associated with primary platinum sensitivity. Seventy-one of 85 (84%) primary carcinomas with an HR mutation (germline or somatic) demonstrated platinum sensitivity. In contrast, 95/158 (60%) carcinomas without an identified HR mutation had platinum sensitivity, and the remainder were platinum resistant or refractory (p=0.0002). Germline HR mutations and somatic HR mutations were each separately predictive of platinum sensitivity compared to cases without HR mutations: 49 of 61 (80%) of cases with a germline mutation were platinum sensitive (p=0.005), and 22 of 24 (92%) carcinomas with a somatic mutation were platinum sensitive (p=0.003, Figure 3). Although platinum sensitivity was correlated with optimal cytoreduction (p<0.00001), carcinomas with HR mutations and those without HR mutations has similar rates of optimal cytoreduction (67% vs 66%, p=0.43).

We assessed whether the observed association with HR mutations and platinum sensitivity was driven by the large number of *BRCA1/2* germline mutations, which have previously been associated with improved survival and platinum responsiveness (5–10). As expected, germline *BRCA1/2* mutations were associated with platinum sensitivity in 38 of 47 (81%) cases (p=0.01, versus no germline or somatic HR mutation). However, the presence of a germline mutation in any non-*BRCA1/2* HR gene or the presence of any HR somatic mutation (including *BRCA1/2*) also predicted platinum sensitivity, with 33/38 (87%) carcinomas exhibiting platinum sensitivity (p=0.002, compared to cases with no germline or somatic HR mutation). The majority of these subjects had somatic *BRCA1/2* mutations. The relatively smaller number of subjects with other HR mutations limits analysis, but 14/18 (78%) carcinomas with a non-*BRCA1/2* HR mutation (germline or somatic) were platinum sensitive, compared to 61% of carcinomas without germline or somatic HR mutations (p=0.14). Subjects who had both a *BRCA1/2* mutation and another HR mutation were excluded from these analyses.

The impact of *PTEN* deficiency on platinum response is unknown. Twelve primary carcinomas had isolated *PTEN* mutations and complete clinical information available; 8 carcinomas (67%) were platinum sensitive. Similarly, 89 of 148 (60%) carcinomas without mutations (no mutations in *PTEN* or HR genes) were platinum sensitive (p=0.8).

Platinum response at recurrence

We assessed whether the presence of an HR mutation in a recurrent carcinoma predicted platinum sensitivity for that recurrence. 45/57 (79%) recurrent carcinomas had complete clinical information allowing determination of platinum sensitivity for that recurrence. Of 29 recurrent carcinomas without germline or somatic HR mutations, only 7 (24%) remained platinum sensitive. Similarly, of 16 recurrent carcinomas with an HR mutation, 5 (31%) remained platinum sensitive (p=0.73). Therefore, HR mutations were more successful at predicting platinum sensitivity at primary treatment than at relapse. However, our recurrent cancers represented a range of clinical scenarios and this question should be re-evaluated in a more uniform setting, such as at first recurrence.

Overall Survival

The presence of a germline or somatic HR gene mutation was associated with significantly better overall survival for women with stage II–IV carcinomas compared to cases without HR mutations (p=0.0006, hazard ratio (HR) 0.6, 95% confidence interval (CI) 0.4–0.8, Figure 4A). The following additional characteristics were significantly related to overall survival: age (p=0.01), optimal versus suboptimal cytoreduction (p=0.001), and stage (p=0.0005). In a multivariate model including these four characteristics, only the presence of an HR mutation (p=0.006) and stage (p=0.0009) remained significantly associated with overall survival, while optimal cytoreduction was of borderline significance (p=0.06) and age was no longer significant (p=0.22). Subjects with germline HR mutations had a median survival of 66 months, compared to 59 months for subjects with somatic HR mutations, and 41 months for subjects without an HR mutation (Figure 4B). Survival in subjects with germline HR mutations was significantly better than subjects without HR mutations (p=0.001). Survival in cases with somatic mutations was similar to germline mutation carriers, but did not reach statistical significance when compared to cases without HR mutations (p=0.09).

Germline *BRCA1/2* mutations were associated with improved overall survival (median 70 months) compared to subjects without HR mutations (p=0.001, HR 0.5, 95% CI 0.4–0.8). Subjects with a germline mutation in HR genes other than *BRCA1/2* or any HR somatic mutation (including somatic *BRCA1/2* mutations) also had improved survival compared to subjects without HR mutations, with a median survival of 59 months vs. 41 months (p=0.05, HR 0.7, 95% CI 0.5–1.0, Figure 4C).

To assess the association of *PTEN* mutations with clinical outcomes, we compared survival in subjects with somatic HR mutations, somatic *PTEN* mutations, and no mutations (no mutations in either *PTEN* or in HR genes). Subjects who had both an HR mutation and a *PTEN* mutation were excluded from analyses. Median overall survival in subjects with *PTEN* mutations was 25.5 months, significantly worse than the 42 months for cases with no mutations (p=0.007, HR= 2.2, 95% CI 1.4–7.4), and 59 months for somatic HR mutations. As only 12 subjects had isolated *PTEN* mutations, these findings require confirmation in larger studies. Although *PTEN* mutation carriers had similar rates of suboptimal cytoreduction compared to cases without mutations (42% vs 31%, respectively), a higher proportion of *PTEN* subjects had stage IV disease (50% vs 11%, p=0.002), which might account for the worse survival. The small number of subjects with *PTEN* mutations preclude multivariate analysis.

Primary-recurrent pairs

Next-generation sequencing can identify somatic mutations that may guide personalized treatment decisions. However, the stability of somatic mutations over time is unclear. It is unknown whether a biopsy should be performed at the time of recurrence to guide treatment decisions, or if archived primary carcinoma tissue could be used. We therefore sought to evaluate neoplastic evolution in paired primary and recurrent ovarian carcinomas based on significant alterations in read ratios of the target mutation. Since comparing the differences in somatic alterations between carcinomas is difficult due to differences in neoplastic purity between samples, we were very conservative in calling differences between the primary and recurrent carcinoma. A pair was considered discordant if a somatic mutation was present in one carcinoma of the pair and absent in the other.

Twenty-three subjects had a paired primary and recurrent carcinoma specimen (Supplementary Table 3). All subjects had at least one mutation present in one or both specimens. Twelve subjects had germline mutations: 8 in *BRCA1*, 2 in *BRCA2*, 1 in *PALB2*,

and 1 in *FAM175A*. Two pairs had somatic HR mutations: one with a somatic *BRCA1* mutation and one with five different somatic mutations in the primary carcinoma. 20 pairs had a somatic *TP53* mutation.

Out of two pairs with somatic HR mutations, one pair was considered concordant. UW124 had a *BRCA1* 3481delA somatic mutation with a similar percent mutant allele in the primary (73%) and recurrent (80%) carcinomas. The pair also had a somatic *TP53* mutation (p.R273C) present in 80% of DNA sequences in the primary and 82% in the recurrent carcinoma. The other pair (UW358) had four different somatic HR mutations (*BRCA1* 1135insA, *BRCA1* 3650insT, *BRIP1* 3260insA, and *MRE11A* 1196insTT), and was considered discordant (Supplementary Table 3). The *BRCA1* 3650insT mutation was present in the recurrent carcinoma (7% mutant allele), but not detected in the primary carcinoma. Whether this represents a new mutation acquired after initial therapy, or whether it was initially present at very low levels at diagnosis and then selected for at recurrence is unknown. The *BRIP1* mutation was present in the primary carcinoma (23% mutant allele), but absent in the recurrent sample.

Twenty pairs had somatic *TP53* mutations. Two pairs were discordant, while 18 were concordant. UW440 had a p.G245D mutation present only in the primary carcinoma, and a c.994(−1)G>A splice site mutation present only in the recurrent carcinoma. A germline *BRCA1* mutation was present in both samples, precluding a sample mix-up. UW406 had a p.R248W mutation detected in 65% of DNA sequences in the primary carcinoma, and was not detected in the recurrent carcinoma.

Nineteen pairs had complete clinical information regarding platinum sensitivity for both the primary and recurrent carcinoma. Recurrent carcinomas with HR mutations developed platinum resistance at a similar rate to those without HR mutations; 3 of 10 recurrent carcinomas (30%) with HR mutations remained platinum sensitive at recurrence compared to 4 of 9 (44%) without HR mutations. However, these comparisons are limited by the heterogeneity of the recurrent carcinomas, which included first recurrences to up to fourth recurrence and intervals from the primary to recurrent carcinoma ranging from 11 to 84 months.

DISCUSSION

Our study demonstrates that both germline and somatic loss-of-function mutations in genes in the FA-BRCA pathway predict higher rates of platinum sensitivity and better overall survival in primary ovarian carcinoma. The improved primary platinum sensitivity associated with germline and somatic HR mutations is consistent with *in vitro* data that cells with defective HR are more sensitive to agents that induce double-strand DNA breaks (15). Women with germline HR mutations had significantly longer overall survival (median 66 months versus 41 months) than subjects without germline or somatic HR mutations (p=0.001), consistent with previous studies demonstrating longer survival in women with germline *BRCA1/2* mutations (5–7, 15). Importantly, somatic *BRCA1/2* mutations and germline and somatic mutations in HR genes other than *BRCA1/2* were also associated with improved survival and platinum sensitivity (p=0.05). To the best of our knowledge, we are the first to correlate the presence of mutations in other HR genes in ovarian carcinomas with clinical outcomes. The improved overall survival observed in germline HR mutation carriers may be due to not only improved response to platinum-based chemotherapy, but also the retention of platinum sensitivity through multiple recurrences, which has previously been reported for *BRCA1/2* carriers (8). We predicted that somatic mutations would be less stable over time due to clonal selection and therefore would have less impact on overall survival than germline mutations. However, overall survival in cases with somatic mutations (median

59 months, HR=0.6) was also improved and was similar to germline mutation carriers, but did not reach statistical significance when compared to cases without HR mutations due to smaller numbers (p=0.09).

Interestingly, subjects with somatic *PTEN* mutations had significantly worse overall survival (median 25.5 months) compared to subjects without any mutations (42 months) or with somatic mutations in HR genes (59 months). *PTEN* mutations also did not correlate with primary platinum sensitivity. Due to the small number of subjects with *PTEN* mutations, these findings require confirmation. The impact of *PTEN* deficiency on homologous recombination is debated. While some studies suggest that carcinoma cells with defective *PTEN* have reduced RAD51-dependent HR and are sensitive to PARPi (16–18), others failed to demonstrate these findings (19, 20). The worse overall survival of cases with *PTEN* mutations in our study suggests that *PTEN* and HR mutations are not similar predictors of outcomes in ovarian carcinomas.

Overall, 31% of ovarian carcinomas had a deleterious germline (24%) and/or somatic (9%) mutation in one of the following 13 HR DNA repair pathway genes: *BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*. We identified mutations in every HR gene included on our panel, and the mutation rate would likely be higher if more genes in the HR pathway were queried. While the majority of both germline and somatic HR mutations were in *BRCA1* or *BRCA2* (74%), 26% occurred in other HR genes. We hypothesize that individuals with mutations (either somatic or germline) in HR genes other than *BRCA1/2* will also have increased response rates to PARPi, as they do to platinum chemotherapy. However, not all genes may be equally important in therapeutic response. Functional assays to determine which of these alterations actually cause PARPi sensitivity, animal models with various genetic defects, and clinical trials which correlate HR mutation status with PARPi response are needed to optimally develop biomarkers of PARPi responsiveness.

Contrary to popular dogma that only high-grade serous ovarian carcinomas are likely to be HR-deficient, we found HR gene mutations (germline and somatic) to be equally common in carcinomas with non-serous histologies. Mutations in HR genes were present in 17 of 61 (28%) non-serous carcinomas and were identified in nearly every histology subtype tested, including clear cell, endometrioid, and carcinosarcoma. While non-serous cases had some *BRCA1/2* mutations, they had a greater proportion of mutations in other HR genes, with two-thirds of germline HR mutations in genes other than *BRCA1/2*. Therefore, non-serous ovarian carcinomas also have a meaningful risk of hereditary breast and ovarian carcinoma, but identification necessitates evaluation with a larger panel of ovarian cancer susceptibility genes. Our findings contrast with those of a recent study of 131 women with non-mucinous ovarian carcinoma, which found that germline *BRCA1/2* mutations were exclusively associated with high-grade serous histology, but evaluated only 23 non-serous cases (21). The identification of three germline *BRCA1/2* mutations in our non-serous cases is unlikely secondary to misclassification, as all of our non-serous cases underwent a recent centralized pathology review by a single gynecologic pathologist blinded to genetic status. Our findings may influence clinical trial design, as most PARPi trials have selected high-grade serous carcinomas as their focus (12). Given the similar HR mutation rate, we suggest that PARPI trials should include a variety of ovarian carcinoma histologies.

It is interesting to compare our germline mutation results with those of the Cancer Genome Atlas (TCGA), which performed exome sequencing in 316 women with high-grade serous ovarian carcinoma (14). Among the 249 women with high-grade serous carcinoma in our series, there were 53 germline *BRCA1/2* mutations, a germline mutation rate of 21%. In contrast, TCGA reported 47 germline *BRCA1/2* mutations in 316 women: 14%, after

subtracting the three reported occurrences of *BRCA2* p.K3326X, a benign polymorphism found in 1% of the general population (22). TCGA's lower germline *BRCA1/2* mutation rate is likely due to cohort selection bias. Many participating IRBs required re-consenting living patients, and thus contributed cases were biased towards deceased patients and new enrollees, but away from long-term survivors. As *BRCA1/2* mutation carriers with ovarian carcinoma have improved overall survival (5–7), the lower number of long-term survivors included in TCGA may have negatively impacted their overall germline *BRCA1/2* mutation rate.

It is more difficult to compare our overall HR deficiency rate with that of TCGA given significant differences in methodology. TCGA reported HR defects in approximately 50% of high-grade serous cases (14), but included a wide variety of genomic alterations which we did not assess, including *BRCA1* hypermethylation, *EMSY* amplification or mutation, and *RAD51C* hypermethylation, which in aggregate comprised 22% of their HR deficiency. *BRCA1* methylation did not impact overall survival in TCGA or in a previous study by our group (23), and the impact of RAD51C methylation is unknown. Furthermore, EMSY amplification, which is thought to silence *BRCA2*, is associated with worse survival, opposite to the expected association for HR deficiency (24, 25). In addition, TCGA assessed many HR genes that we did not assess, counted all missense mutations as deleterious, and included somatic *PTEN* mutations as HR-deficient. Furthermore, TCGA did not assess germline mutations in HR genes other than *BRCA1/2*. Therefore, other than the cases with germline and somatic *BRCA1/2* mutations, we have likely identified a different subset of ovarian carcinomas to be HR-deficient than were classified as such by TCGA.

We analyzed 23 paired primary and recurrent ovarian carcinomas in order to evaluate the stability of somatic mutations over time. The vast majority of somatic *TP53* mutations were concordant, although two were not. *TP53* is thought to be a driver event in ovarian carcinogenesis, and it is possible that *TP53* mutations are more stable over time compared to other mutations. As only two paired cases had somatic HR mutations, we are unable to generalize on the stability of HR somatic mutations during treatment. We and others have shown that germline *BRCA1/2* mutations can "revert" to wildtype sequence in recurrent ovarian carcinoma (26–30), and we presume somatic HR mutations would be under a similar high negative selection pressure during multiple rounds of chemotherapy. Obtaining tissue biopsies of recurrent ovarian carcinoma at uniform time-points in the treatment setting are critical to understanding clonal progression. These studies would determine when obtaining a biopsy at recurrence is needed and when archived primary carcinoma tissue can be used to guide personalized treatment decisions.

In summary, germline and somatic mutations in HR genes are present in almost one-third of ovarian carcinomas and predict a better response to primary platinum chemotherapy and improved overall survival. We hypothesize that individuals with these mutations will also have increased response rates to PARPi. Clinical trials of PARPi which fully characterize genetic status will be needed to confirm this hypothesis. Notably, non-serous ovarian carcinomas have an equal rate of HR mutations relative to serous carcinomas, but with a higher fraction of those mutations in genes other than *BRCA1/2*.

MATERIALS AND METHODS

Study Subjects

Women with ovarian, fallopian tube, or primary peritoneal carcinoma who underwent surgery at the University of Washington, Seattle, WA or at Swedish Hospital, Seattle, WA, and provided informed consent approved by the human subjects divisions of the institutional review board were eligible for the study. Subjects were prospectively enrolled at diagnosis

and not selected for age or family history. We excluded carcinomas identified at the time of risk-reducing surgery performed due to genetic risk. Clinical information was retrieved from medical records. Genomic DNA was extracted from peripheral blood mononuclear cells (germline DNA) and from frozen or formalin fixed paraffin embedded (FFPE) sections from areas with 60% or greater neoplastic cellularity. Library construction, hybridization, and massively parallel sequencing was performed as previously described (1). The 30 gene panel (Supplementary Table 4) was designed to include all known breast and ovarian cancer genes, as well as additional HR-related genes most integral to the FA-BRCA pathway. Assessment for germline mutations was previously reported for 216 subjects in 21 genes (1); these cases were assessed for 9 additional genes using the 30 gene panel. A small subset of samples previously underwent testing for germline mutations in other HR genes (such as *FAM175A* and *CHEK1*), and when these were identified, they were also reported. Additionally, a total of 243 subjects had germline (lymphocyte) DNA assessed for mutations in *RAD51D*, either through Sanger sequencing (reported previously in 216 subjects (31)) or through targeted capture and genomic sequencing (27 subjects).

All cases with non-serous histology were reviewed by a dedicated gynecologic pathologist (MR). Cases with high-grade endometrioid histology as well as those with mixed or uncertain histology were also reviewed by a second gynecologic pathologist (RG) and a consensus diagnosis was obtained for each case. Cases with mixed histology were only considered non-serous if the predominant histology (>50%) was not serous.

Mutation Analysis

The BROCA panel identifies all classes of mutations, including single-base substitutions, small insertions and deletions, and large gene rearrangements (32). Sequence alignment and variant calling were performed against the reference human genome (UCSC hg19) as previously described (1). Each variant was annotated with respect to gene location and predicted function in HGVS nomenclature. *BRCA1* and *BRCA2* mutations were annotated using the designations used by the Breast Cancer information Core (BIC, http://research.nhgri.nih.gov/bic/, BRCA1 GenBank U14680; BRCA2 GenBank U43746); all other mutations were annotated using HGVS nomenclature. Deletions and duplications of exons have been detected in normal cells by a combination of relative read depth and split read algorithms, as described previously (32, 33). For carcinoma samples, changes in copy number state were identified using similar normalized read depth approach, but incorporating normal mixture modeling via expectation maximization (34), which allowed detection of copy number state changes that were present in a proportion of the complex population of cells in the tumor sample. Using this approach, we were able to detect amplification and deletion of entire genes and of small CNVs within the loci down to single exon resolution (approximately 200bp).

For all suspected loss-of-function variants, PCR amplification and Sanger sequencing was performed both on lymphocyte-derived (germline) and neoplastic DNA to confirm and classify the mutation as somatic or germline. Only missense variants previously demonstrated to be deleterious were included. There was no minimum threshold for the variant reads for somatic mutations as long as they validated with Sanger sequencing. For somatic large gene rearrangements or copy number variations (CNVs), any gene-disrupting intragenic deletion or duplication was considered deleterious. Homozygous whole gene deletions were considered deleterious; hemizygous whole gene deletions (i.e loss of heterozygosity) were excluded. CNVs were validated using PCR amplification and Sanger sequencing when break points could be identified. If breakpoints were not clear, CNVs were validated using quantitative PCR.

Mutation analysis was performed on the paired primary and recurrent carcinomas using an alternate pipeline tailored to detect somatic mutations in clinical cancer specimens, as described by Pritchard et al (35). The percent of mutant allele present in DNA sequences was compared in the paired primary and recurrent carcinoma using read ratios (variant reads/total reads) and also using fluorescent peak ratios from Sanger sequencing for each target mutation. Each primary-recurrent pair was classified as concordant or discordant based on alterations in read ratios of the target mutation between the primary and recurrent carcinoma. Read ratios were only used for comparison when both carcinomas had adequate total number of reads (>100); in two cases with poor depth of coverage, fluorescent peak ratios from Sanger sequencing were used instead. A pair was considered discordant if a mutation was present in only one carcinoma of the pair and absent in the other.

Statistical Analysis

In cases with paired primary and recurrent samples, individuals were counted only once and the primary carcinoma of the pair was used for analysis (unless otherwise specified). When subjects had both a germline and a somatic HR mutation, they were included in the germline HR mutation group and not the somatic mutation group for analyses. Significance of contingency tables was analyzed with Chi-squared or Fisher's exact test. Primary platinum sensitivity was defined by a complete response during adjuvant chemotherapy and clinical remission for at least 6 months after completion of chemotherapy. Primary platinum resistance was defined as progressive disease on platinum therapy, less than a complete response to platinum therapy, or progression within 6 months of completing platinum therapy. To classify platinum responsiveness in recurrent carcinomas, the actual response to platinum-based chemotherapy for that recurrence was used, and not the previous interval since previous chemotherapy. If the subject's most recent treatment did not include platinum and if her previous interval between treatment and progression was ≥6 months, then she was considered non-evaluable for platinum sensitivity. If that interval was <6 months or if she was previously classified as platinum resistant, then that recurrence was considered platinum resistant.

Survival analyses were performed using the methods of Kaplan and Meier; differences were assessed using the log-rank test. Significant variables for survival were used as covariates in a multivariate model. Overall survival was calculated from time of diagnosis to death. Survival data was censored for living patients at time of last follow-up.

All *P*-values were two-tailed, with alpha set at 0.05. GraphPad Prism software (La Jolla, CA) was used for all statistical analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support: Supported by NIH/NCI grants R01CA131965 (ES), P50CA083636 (ES), and R01CA157744 (TW, M-CK), the Ovarian Cancer Research Fund (ES), Wendy Feuer Ovarian Cancer Research Fund (ES), the Cori and Tony Bates Novel Technologies for Cancer Prevention Fund (ES), and the Department of Defense Ovarian Cancer Research Program OC093285 (TW).

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Statement of translational relevance

We have demonstrated that germline and somatic loss-of-function mutations in homologous recombination (HR) DNA repair genes are associated with significantly improved primary platinum sensitivity and overall survival. These better outcomes applied to somatic as well as germline mutations and to genes other than *BRCA1* and *BRCA2*. Until now, many scientists assumed that HR deficiency occurs more commonly in high-grade serous ovarian carcinomas. However, we found that HR mutations occurred at similar frequency in non-serous carcinomas. It is known that ovarian carcinomas associated with germline *BRCA1* and *BRCA2* mutations have high response rates to treatment with PARP inhibitors. We hypothesize that ovarian carcinomas with mutations in HR genes other than *BRCA1* and *BRCA2* will also respond to PARP inhibitors and suggest that PARP inhibitor trials should target both non-serous and serous carcinomas.

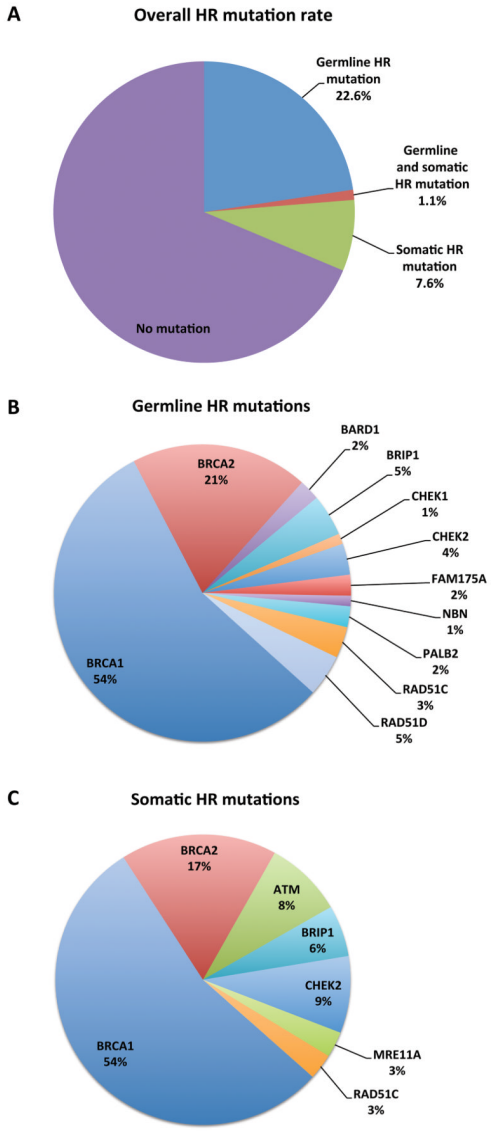

Figure 1.

Mutation rates in HR genes. **A**, Overall, 115 of 367 subjects (31.3%) had deleterious mutations in 13 HR genes: 83 (22.6%) with germline HR mutations, 28 (7.6%) with somatic HR mutations, and 4 (1.1%) with both germline and somatic HR mutations. Mutations were detected in every HR gene tested. **B**, Eighty-seven subjects (24%) had 88 germline mutations in 11 HR genes. Germline HR mutations included 49 (13.4%) in *BRCA1*, 17 (4.6%) in *BRCA2*, and 22 (6%) in other HR genes, including *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*. **C**, Thirty-two carcinomas (8.7%) had a total of 35 somatic mutations in 7 HR genes. Somatic HR mutations included 19 (5.2%) in *BRCA1*, 6 (1.6%) in *BRCA2*, and 10 (2.7%) in other HR genes, including *ATM*, *BRIP1*, *CHEK2*, *MRE11A*, and *RAD51C*.

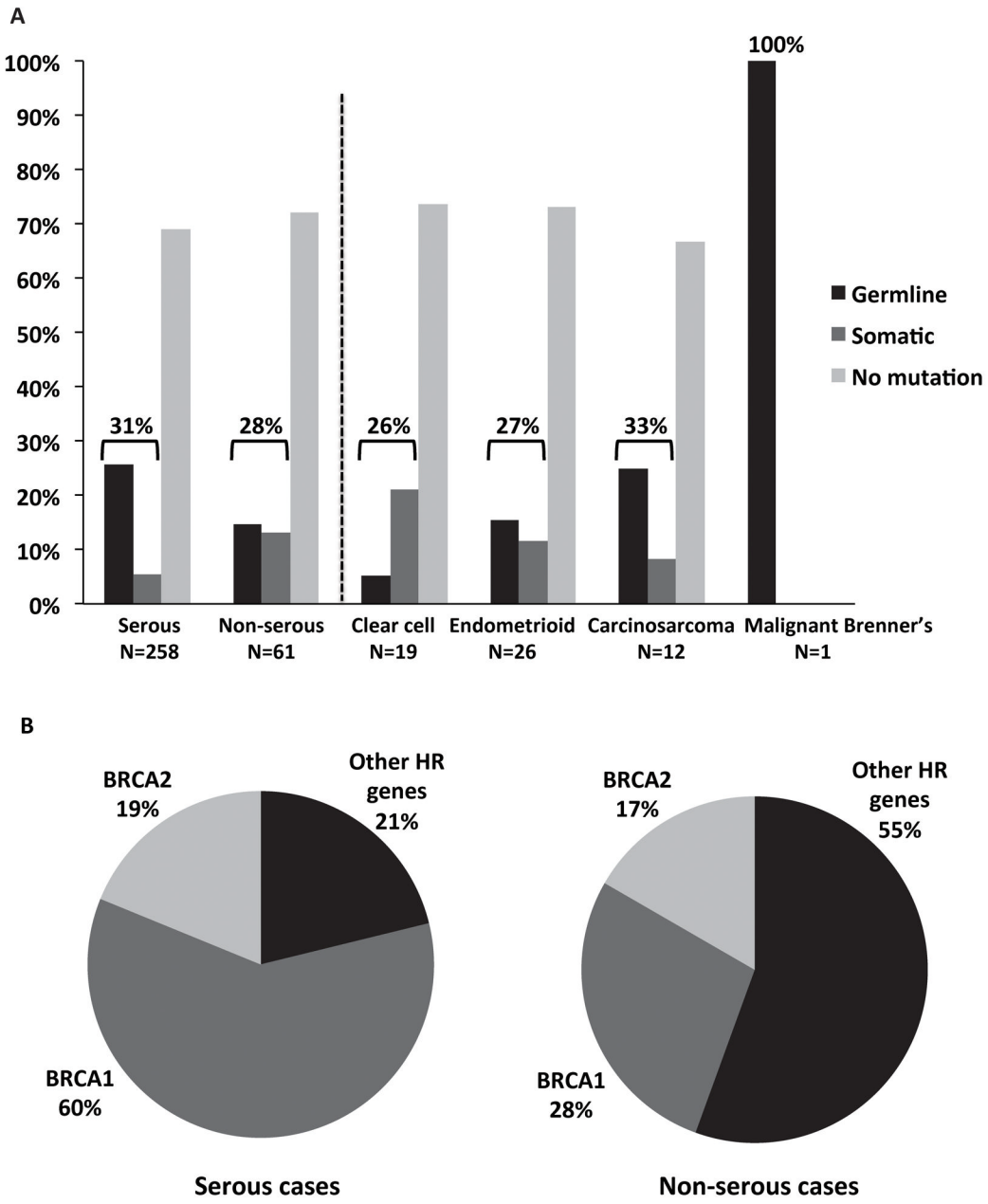

Figure 2.

HR gene mutation rates by histology. **A**, HR gene mutations were identified at similar frequencies in non-serous and serous carcinomas. Seventeen of 61 (28%) non-serous cases and 80 of 258 (31%) serous cases had a deleterious germline or somatic HR mutation (p=0.63). HR gene mutations were identified in almost every type of non-serous histology tested, including 5/19 (26%) clear cell, 7/26 (27%) endometrioid, 4/12 (33%) carcinosarcoma, and 1/1 (100%) malignant Brenner's carcinoma. No mutations were identified in the one mucinous or two mixed histology carcinomas. **B**, Whereas serous carcinomas had a predominance of mutations in *BRCA1* and *BRCA2*, the non-serous carcinomas had a much wider distribution of mutations in other HR genes. In non-serous carcinomas with HR mutations, 56% of mutations were in other HR genes, compared to only 21% of mutations in serous carcinomas (p=0.005).

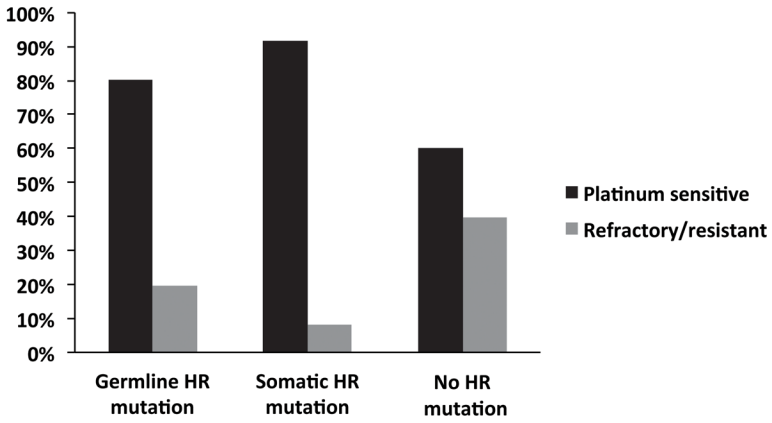

Figure 3.

Germline HR mutations and somatic HR mutations were each predictive of platinum sensitivity compared to cases without HR mutations: 49 of 61 (80%) cases with a germline mutation (p=0.008), and 22 of 24 (92%) carcinomas with a somatic mutation (p=0.003) were platinum sensitive (p=0.003). In contrast, only 95 of 158 (60%) carcinomas without an identified HR mutation had primary platinum sensitivity.

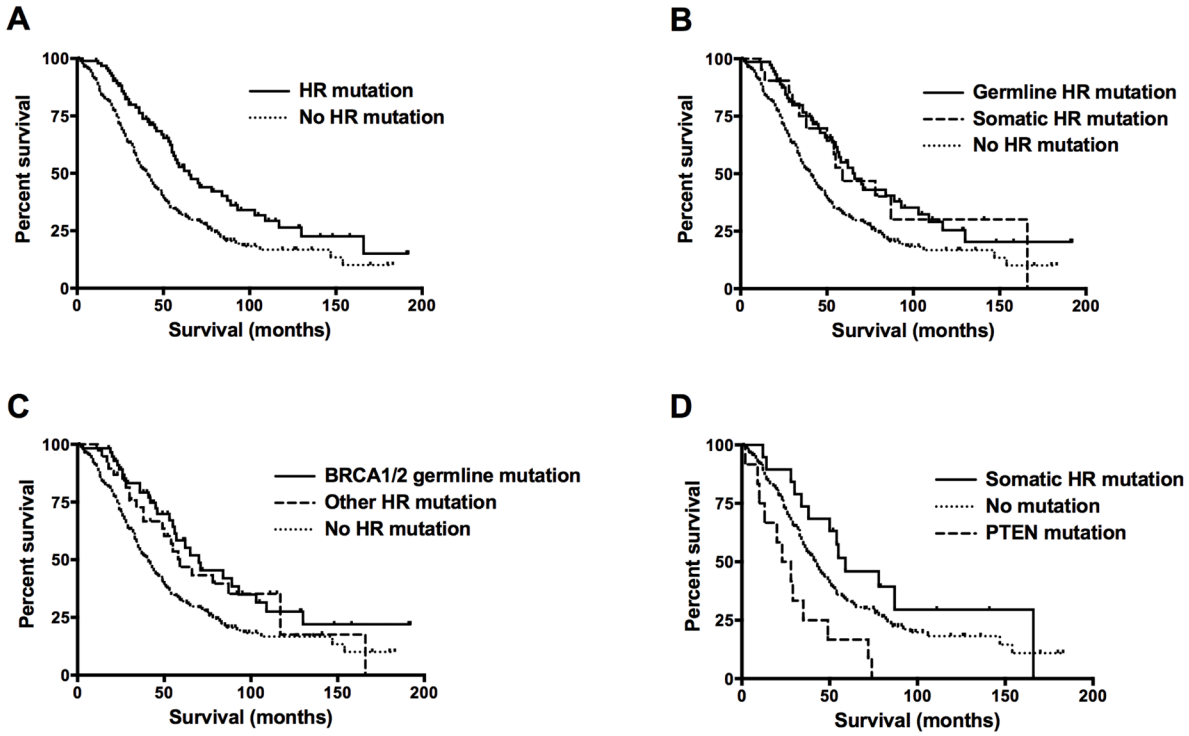

Figure 4.

Overall survival by genetic status. **A**, The presence of an HR gene mutation was associated with an improved overall survival compared to cases without HR mutations (median overall survival 66 vs. 41 months, p=0.006, hazard ratio (HR) 0.6, 95% confidence interval (CI) 0.4 to 0.8). HR gene mutations were significantly related to overall survival after accounting for the covariates age, stage, and optimal cytoreduction (p=0.006). **B**, Overall survival in subjects with germline HR mutations was significantly better than subjects without HR mutations (median 66 months vs. 41 months, p=0.001). Overall survival in cases with somatic mutations (median 59 months) was similar to germline mutation carriers, but these differences did not reach statistical significance when compared to cases without HR mutations (p=0.09). **C**, Subjects with germline *BRCA1/2* mutations had improved overall survival compared to subjects without HR mutations (median 70 months vs. 41 months, p=0.001, HR 0.5, 95% CI 0.4 to 0.8). Subjects with a germline mutation in HR genes other than *BRCA1/2* or any HR somatic mutation (including *BRCA1/2* mutations) also had improved survival (median 59 months, p=0.05, HR 0.7, 95% CI 0.5 to 1.0). **D**, Median overall survival in subjects with *PTEN* mutations was 25.5 months, significantly shorter than for cases with no HR or *PTEN* mutations (median 42 months, p=0.007, HR= 2.2, 95% CI 1.4 to 7.4), and cases with somatic HR mutations (median 59 months).

Table 1

Clinical characteristics and fraction with HR mutations.

	All Subjects N	Fraction with Germline HR mutation[a]	Fraction with Somatic HR mutation[a]
Median age (years)	59 y	53 y	59 y
Range	(27–89 y)	(34–75 y)	(29–80 y)
Site			
Ovary	304	.22	.08
Fallopian tube	24	.42	.04
Peritoneal	32	.22	.03
Synch Ov/Endo	7	.29	.14
Histology			
High-grade[b] serous	249	.26	.05
Low-grade[c] serous	9	.11	0
Poorly-differentiated NOS	48	.25	.13
Clear cell	19	.05	.21
High-grade[b] endometrioid	20	.15	.10
Low-grade[c] endometrioid	6	.17	.17
Carcinosarcoma	12	.25	.08
Other[d]	4	.25	0
Stage[e]			
I	36	.17	.14
II	19	.16	0
III	255	.24	.08
IV	49	.27	.04
Cytoreduction[e]			
Optimal	243	.23	.09
Suboptimal	109	.22	.06
Total	367	.24	.08

[a] Cases with both a germline and somatic HR mutation were included in the germline HR category

[b] Grade 2–3

[c] Grade 1

[d] Other = one malignant Brenner's, one mucinous, and two mixed carcinomas

[e] stage was unknown for 8 cases

[f] cytoreduction status was not available for 15 cases

Synch Ov/Endo: cases classified pathologically as having two primary cancers arising from the ovary and endometrium

NOS: not otherwise specified

Table 2

Non-serous cases with HR mutations.

Histology	ID	Grade	Germline HR mutation[a]	Somatic HR mutation(s)[a]	Somatic *PTEN* mutation(s)[a]
Clear cell	UW400	3	*CHEK2* p.S428F		
	UWf14	3		*BRCA2* p.S368X	
	UW420	3		*CHEK2* del exon 1–7	
	UW408	3		*ATM* c.5441delT	*PTEN* c.678delC
	UW358	3		*BRCA1* 1135insA *BRIP1* c.3260insA *MRE11A* c.1196insTT	*PTEN* c.968insA
Endometrioid	UW383	1	*BRIP1* p.R798X		*PTEN* c.955delACTT
	UW381	1		*ATM* c.3284(+1)G>C splice	
	UW131	2		*BRCA2* p.R2494X	
	UW341	2		*BRCA1* dup exon 21–24	
	UW165	3	*BRCA1* 187delAG		
	UWf2	3	*BRCA2* 3034delAAAC		
	UW132	3	*RAD51D* c.580delA		*PTEN* c.389delG
Carcinosarcoma	UWf77	3	*BRCA1* 2080delA		
	UW435	3	*FAM175A* c.1106insG		
	UW407	3	*RAD51C* c.706(−2) A>G splice		
	UW124	3		*BRCA1* 3481delA	
Malignant Brenner's	UW96	3	*FAM175A* c.1106insG		

[a] *BRCA1* and *BRCA2* mutations were annotated using BIC designation (http://research.nhgri.nih.gov/bic/, reference sequences: *BRCA1* GenBank U14680; *BRCA2* GenBank U43746); all other mutations were annotated using HGVS nomenclature.