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Pathogenic germline variants in non-*BRCA* homologous recombination genes in ovarian cancer: analysis of tumor phenotype and survival

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Abstract

Objective: To define molecular features of ovarian cancer (OC) with germline pathogenic variants (PVs) in non-*BRCA* homologous recombination (HR) genes and analyze survival compared to *BRCA1/2* and wildtype (WT) OC.

Methods: We included patients with OC undergoing tumor-normal sequencing (MSK-IMPACT) from 07/01/2015-12/31/2020, including germline assessment of other HR genes *ATM, BARD1, BRIP1, FANCA, FANCC, NBN, PALB2, RAD50, RAD51B, RAD51C, and RAD51D*. Biallelic inactivation was assessed within tumors. Progression-free (PFS) and overall survival (OS) were calculated from pathologic diagnosis using the Kaplan-Meier method with left truncation. Whole-exome sequencing (WES) was performed in a subset.

Results: Of 882 patients with OC, 56 (6.3%) had germline PVs in non-*BRCA* HR genes; 95 (11%) had BRCA1-associated OC (58 germline, 37 somatic); and 59 (6.7%) had *BRCA2*-associated OC (40 germline, 19 somatic). High rates of biallelic alterations were observed among germline PVs in *BRIP1* (11/13), *PALB2* (3/4), *RAD51B* (3/4), *RAD51C* (3/4), and *RAD51D* (8/10). In cases with WES (27/35), there was higher tumor mutational burden (TMB; median 2.5 [1.1-6.0] vs. 1.2 mut/Mb [0.6-2.6]) and enrichment of HR-deficient (HRD) mutational signatures in tumors associated with germline *PALB2* and *RAD51B/C/D* compared with *BRIP1* PVs ($p<0.01$), although other features of HRD, including telomeric-allelic imbalance (TAI) and large-scale state transitions (LSTs), were similar. Although there was heterogeneity in PFS/OS by gene group, only *BRCA1/2*-associated OC had improved survival compared to WT OC ($p<0.01$).

Conclusions: OCs associated with germline PVs in non-BRCA HR genes represent a heterogenous group, with *PALB2* and *RAD51B/C/D* associated with an HRD phenotype.

Keywords

ovarian cancer; homologous recombination; *BRCA1/2*; poly (ADP-ribose) polymerase; inhibition; survival; genetics

Introduction

Ovarian cancer (OC), specifically high-grade serous ovarian cancer (HGSOC), is characterized by deficiencies in homologous recombination (HR).[1] Patients with germline (g) and somatic (s) *BRCA1/2* pathogenic variants (PVs), genes that play a critical role in HR, are associated with improved survival[2,3] compared to those without PVs in *BRCA1/2* or other HR genes, which we termed wildtype (WT) tumors. The former also derive clinical benefit from platinum-based therapies and targeted therapies such as poly (ADP-ribose) polymerase (PARP) inhibitors.[4,5]

Germline PVs in genes other than *BRCA1/2* associated with HR are also found in OC and HGSOC, and many, including *RAD51C, RAD51D, BRIP1*, and *PALB2*, have been associated with an increased risk of OC development, albeit to a lesser extent than *BRCA1/2*.[6] The genomic landscape and HR signatures in these tumors as well as the survival outcomes of these patients compared to those with *BRCA*1/2-associated and WT OC is unknown.

We sought to comprehensively characterize the genomic landscape and measures of HR-deficiency (HRD) in OCs of patients with germline PVs in non-*BRCA1/2* HR genes and evaluate clinical outcomes and survival compared to patients with g/s *BRCA1/2*-associated and WT tumors.

Methods

Patient Selection

We included all patients treated at our institution with pathologically confirmed epithelial OC who underwent clinical tumor-normal sequencing using MSK-IMPACT (Memorial Sloan Kettering Cancer Center – Integrated Mutation Profiling of Actionable Cancer Targets), targeting 341-505 cancer-related genes and inclusive of germline analysis of ≥76 genes,[7,8] between 07/01/2015 and 12/31/2020. Universal germline and somatic genetic testing are recommended in OC.[9] Our institution utilizes MSK-IMPACT to accomplish this, and testing is offered to all patients in an unselected manner. This study was approved by the Institutional Review Board of MSK (IRB protocol 12-245).

Genetic Analysis

PVs were independently assessed and manually curated using standards for variant classification by trained molecular pathologists.[10] Variants of uncertain significance were not included. Patients and tumors with *BRCA1/2* PVs were identified, as were those with germline PVs in other HR genes, including *ATM, BARD1, BRiP1, FANCA, FANCC, NBN, PALB2, RAD50, RAD51B, RAD51C*, and *RAD51D*.[11,12] Genes were chosen based on

prior studies and availability on germline panels.[6,13,14] For germline PVs in HR genes, biallelic inactivation was inferred through assessments of loss of heterozygosity (LOH) within tumors at the germline variant locus using the FACETS (fraction and allele-specific copy number estimates from tumor sequencing) algorithm[15] and evaluation of secondary somatic mutations. These patients were separated into two groups—those with ≥60% LOH (high) and those with <60% LOH (low)—based on prior reports in *BRCA1/2* germline PVs across all cancer types.[16]

Data Collection

Clinicopathologic data were abstracted from the medical record and included date of pathological diagnosis, self-reported race/ethnicity, PARP inhibitor therapy, and clinical HRD testing (myChoice®, Myriad Genetics, Salt Lake City, UT, USA).[17] Age was measured at date of pathological diagnosis. Histology was abstracted from pathological reports and stratified by HGSOC or other. Stage was defined at pathological diagnosis using the 2014 International Federation of Gynecology and Obstetrics (FIGO) staging system.[18] Initial treatment was categorized as primary debulking surgery (PDS) or neoadjuvant chemotherapy (NACT) with plans for interval debulking surgery (IDS). A complete gross resection (CGR) was defined as no visible residual disease at the completion of surgery. Body mass index (BMI) was calculated at diagnosis, and obesity was defined as BMI ≥30 kg/m^2. Smoking was defined as ever vs. never smoker.

Statistical Analysis

Clinical characteristics were analyzed using summary statistics and reported by overall and genetic status: (g/s) *BRCA1*, (g/s) *BRCA2*, other HR, and WT (not classified into the other groups). Comparisons between gene groups were made using non-parametric tests. Progression-free survival (PFS) and overall (OS) survival were defined as time from pathological diagnosis to clinical progression or radiographic progression and time to death or last follow-up in those still living, respectively. A left truncation method was used to account for the time gap from diagnosis date to MSK-IMPACT consent date and to reduce possible biases. This excludes the subset of patients who may have undergone MSK-IMPACT genetic testing in the recurrent setting and limits survival analyses to those who underwent genetic testing close to diagnosis of OC. Kaplan-Meier survival analysis was used to estimate median PFS and OS by gene group. A multivariable Cox proportional hazards (PH) model with left truncation was created to examine the relationship between PFS/OS and gene groups, adjusting for covariates. For variables occurring after the date of pathological diagnosis (eg, CGR), landmark analysis was used.

Whole-Exome Sequencing

In those with germline PVs in other HR genes and high LOH, whole-exome sequencing (WES) was performed from available tumor samples and matched normal samples. Data analysis was performed as previously described.[19] Tumor mutational burden (TMB) was calculated by dividing the number of nonsynonymous mutations by the total size of the capture panel in Mb. To define the mutational signatures, we assessed the mutational context of synonymous and nonsynonymous single nucleotide variants (SNVs) in samples subjected to WES employing SigProfiler[20] and SIGNAL.[21] Based on the copy number amplifications

(CNAs) by FACETS, the fraction of genome altered (FGA) was defined as the length of segments with log2 or linear CNA value larger than 0.2 divided by the length of all segments measured. Large-scale state transition (LST) scores, defined as chromosomal breakpoints resulting in allelic imbalance between adjacent regions of at least 10Mb, were determined.[22-25]

Results

Gene Groups

Of 1266 patients with epithelial OC who underwent tumor-normal sequencing during the study period, 882 were treated at MSK from diagnosis and were included in these analyses. Among these 882 patients, 95 (11%) had a *BRCA1* PV (58 *gBRCA1* and 37 *sBRCA1*); 59 (6.7%) had a *BRCA2* PV (40 g*BRCA2* and 19 s*BRCA2*); and 672 (76%) had WT OC. We observed germline PVs in non-*BRCA* HR genes in 56 patients (6.3%; Figure 1).

Patient Characteristics

Mean age at diagnosis for the entire cohort was 63 years (range, 21-93) with variation by gene group. Primary surgery was performed in 515/882 patients overall (58%), and 604 (80%) of 752 patients with known residual disease status achieved a CGR with PDS or IDS, with no differences between gene groups. Eighty-five percent of patients were diagnosed with stage III/IV disease, with some variation among gene groups. High-grade serous histology comprised 77% of OCs overall, with higher rates among those with *BRCA1/2* PVs. Seventy-seven percent identified as White, with no differences in race, ethnicity, or smoking status among gene groups (Table 1).

Somatic Landscape of Tumors in Patients with Germline PVs in non-BRCA HR Genes

We observed heterogeneity of germline-somatic interactions within tumors from patients with germline PVs in non-*BRCA1/2* HR genes. High rates of biallelic inactivation and LOH were found within tumors from those with germline PVs in *BRIP1* (85%, 11/13), *PALB2* (75%, 3/4), *RAD51B* (75%, 3/4), *RAD51C* (75%, 3/4), and *RAD51D* (75%, 8/10), a group we designated as “Other HR, LOH High”. In contrast, low rates of LOH were observed within tumors from those with germline PVs in *ATM* (29%, 2/7), *NBN* (25%, 1/4), *BARD1* (0%, 0/2), *FANCA* (0%, 0/2), *FANCC* (50%, 1/2) and *RAD50* (0%, 0/4), a group we designated as “Other HR, LOH Low” (Figure 2).

Among the 35 OCs with high LOH, WES was performed in 27 tumors with adequate tumor purity (≧30%) (*BRIP1*, n=11; *PALB2*, n=2; *RAD51B*, n=3; *RAD51C*, n=2; *RAD51D*, n=9) (Figure 1). Twenty-five (93%) of the 27 tumors exhibited LOH of the WT allele, and 2 (7.4%) showed no LOH or second somatic mutation. We observed heterogeneity among tumors with enrichment of mutational signatures related to HRD in tumors associated with germline PVs in *PALB2* and *RAD51B/C/D* compared with *BRIP1* ($p<0.01$; Figure 3). TMB was also higher in tumors associated with germline PVs in *PALB2* and *RAD51B/C/D* compared with *BRIP1* (median, 2.5 mut/Mb [range, 1.1-6.0] vs. 1.2 mut/Mb [range, 0.6-2.6]; $p<0.001$). Genomic features of HRD, including telomeric-allelic imbalance (TAI) and LSTs, as well as chromosomal instability as defined by FGA, were similar in

tumors associated with germline *PALB2, RAD51B/C/D*, and *BRIP1* PVs (Figure 3 and Supplementary Figure 1).

Based on these data, those with germline PVs in non-*BRCA* HR genes were further stratified into three groups: 1) Other HR, HRD, 2) Other HR, no HRD, and 3) Other HR, LOH Low with distinct clinical features (Supplementary Table 1).

Survival Outcomes

Among the 705 patients included in the survival analysis due to left truncation, there were 304 events of progression/ recurrence, with 58 deaths without progression. Median follow-up was 35.2 months (range, 0.4-195.6 months). Given potential differences between somatic and germline *BRCA1/2* PVs and heterogeneity within tumors found in those with germline PVs in other HR genes, we performed survival analyses (PFS and OS) in five groups (*BRCA1, BRCA2*, Other HR LOH High, and Other HR LOH Low compared to WT) and eight groups (*gBRCA1, sBRCA1, gBRCA2, sBRCA2*, Other HR HRD, Other HR No HRD, and Other HR LOH Low compared to WT) and found significant variations (Figure 4 and Supplementary Figure 2).

Among the five groups on univariate analysis, PFS was improved for patients with OCs associated with *BRCA1* (hazard ratio, 0.39; 95% CI: 0.26-0.58) and *BRCA2* (hazard ratio, 0.32; 95% CI: 0.17-0.58) PVs. We observed no significant differences in those with germline PVs in other HR genes compared to those with WT tumors. However, the curves did separate by LOH status, and the PFS curve for those with high LOH clustered with curves from those with *BRCA1/2*-associated OC (Figure 3A and Supplementary Table 2). Similar findings were observed on univariate analyses of OS for those with *BRCA1* (hazard ratio, 0.38; 95%: 0.22-0.65) and *BRCA2* (hazard ratio, 0.48; 95% CI: 0.27-0.87) PVs, who had improved OS. Again, there were no significant differences between those with germline PVs in other HR genes compared to WT-associated OCs; however, a similar separation of the curves by LOH levels was observed (Supplementary Figure 2A and Supplementary Table 3).

Among the eight gene groups on univariate analysis, OC associated with somatic and germline PVs in *BRCA1/2* exhibited better PFS and OS than WT-associated OC, with the lowest hazard ratio in those with *sBRCA2* PVs (PFS hazard ratio, 0.15; 95% CI: 0.04-0.6 and OS hazard ratio, 0.13; 95% CI: 0.02-0.93). The PFS curve of OCs with germline PVs in other HR genes that exhibited high LOH and HRD phenotype clustered with curves of *BRCA1/2*-associated OCs, with a hazard ratio suggesting improved PFS (hazard ratio, 0.34; 95% CI: 0.11-1.06). However, this was not significantly different in comparison to those with germline PVs in other HR genes with low LOH or no HRD phenotype and WT-associated OC. OS did not differ significantly between those with germline PVs in other HR genes and WT OC, although a similar separation of the OS curves of those with germline PVs in other HR genes was observed by LOH and HRD status (Figure 3B, Supplementary Figure 2B, and Supplementary Table 4).

Notably, within the WT group there were only 3 cases with somatic mutations in *BRIP1, PALB2, or RAD51B/C/D*, and all were monoallelic. Additionally, PDS, younger age and

earlier stage at diagnosis, non-high-grade serous histology, and CGR were associated with better PFS (p<0.001) and OS (p<0.001) (Supplementary Tables 2 and 3).

Multivariable Models

Multivariable models of survival using left truncation and landmark analysis were fitted among the five gene groups and incorporating other clinical variables. *BRCA1/2* PVs were associated with improved PFS and OS, even after adjustment for initial treatment (PDS vs. NACT), stage at diagnosis, and CGR rates. Although the hazard ratios for PFS (hazard ratio, 0.55; 95% CI: 0.28-1.08) and OS (hazard ratio, 0.68; 95% CI: 0.35-1.30) were lower for those with germline PVs in other HR genes with high LOH, it was not significantly different from those with low LOH and WT OC (Supplementary Table 5).

Clinical HRD Testing and PARP Inhibitor Therapies

Among all patients with germline PVs in non-BRCA HR genes, 12 (21%) of 56 underwent clinical HRD testing of their tumors; 44 (79%) of 56 did not. Among those who underwent HRD testing, 4 (33%) of 12 had OCs that exhibited an HRD phenotype. These were associated with germline PVs in *PALB2* (n=1), *ATM* (n=1), *FANCA* (n=1), and *RAD51D* (n=1). Of these, tumors associated with germline PVs in *PALB2/RAD51D* also underwent WES and exhibited an HRD phenotype. Additionally, 7 (58%) of 12 were HR proficient and 1 (8.3%) of 12 had inconclusive results on clinical testing (Supplementary Table 6).

Twenty-eight (50%) of 56 patients received PARP inhibitor therapy—5 (18%) of 28 in first-line as maintenance; 9 (32%) of 28 in second-line as maintenance; and 14 (50%) of 28 in third-line or beyond (treatment in 5 patients and maintenance in 9 patients). PARP inhibitor therapy was used for 6 (46%) of 13 patients with *BRIP1*, 3 (75%) of 4 with *PALB2*, 2 (50%) of 4 with *RAD51B*, 3 (75%) of 4 with *RAD51C*, and 8 (80%) of 10 with *RAD51D* germline PVs (Supplementary Table 6). Detailed information on response to therapy was not available.

Discussion

We explored the molecular and clinical features of OC from patients with germline PVs in other HR genes and compared them with *BRCA1/2*-associated and WT OCs. Rates of biallelic alterations differed, with high rates among germline PVs in *BRIP1, PALB2, RAD51B, RAD51C*, and *RAD51D*; however, HRD phenotype varied within this group, with enrichment in *PALB2* and *RAD51B/C/D* compared to *BRIP1*. PFS and OS varied by gene group, with best survival among *BRCA1/2*-associated OCs, even after adjustment for clinical covariates in multivariable models. Although patients with germline PVs in other HR genes did not have significantly better survival than those with WT OC, we observed heterogeneity in PFS and OS by biallelic status and HRD phenotype. Larger studies should explore these findings to aid in selection of targeted therapies and precision medicine.

Our findings are consistent with emerging evidence suggesting germline PVs in non-*BRCA* HR genes, particularly *PALB2, RAD51C*, and *RAD51D*, confer an HRD phenotype in OC, are associated with improved outcomes, and potentially serve as predictive biomarkers for PARP inhibitor response.[2,3,26-29] Although high rates of biallelic inactivation at *BRCA1/2*

germline PVs, HRD phenotype, and associations with favorable prognosis and response to PARP inhibitor therapy have been previously reported across cancer types and in OC,[2,16] very little is known about germline PVs in other HR genes.

In an exploratory analysis from ARIEL2, a trial of rucaparib treatment for recurrent OC,[30] Swisher et al. identified 7 of 491 patients with *RAD51C/D* germline PVs who benefited from rucaparib, with an overall response rate (ORR) of 71.4% (95% CI: 29.0-96.3). However, there were no patients with *PALB2* germline PVs, and response in patients with alterations in other HR genes, including *BRIP1*, was not different from that of WT patients (ORR, 3.4; 95% CI: 0.1-17.9).[30] *In vitro* studies have also suggested that pathogenic defects in *BRIP1* may not confer benefit to PARP inhibitor therapy.[31] Similarly, in an exploratory analysis of 806 patients from the PAOLA-1 study, a randomized phase III trial of maintenance bevacizumab with or without olaparib in newly diagnosed OC, Pujade-Lauraine et al. identified germline PVs in non-*BRCA1/2* HR genes in 3.7-9.8% of patients, depending on the multigene panel used. Although germline PVs in all non-*BRCA1/2* HR genes were not predictive of PFS benefit to olaparib with bevacizumab, there were high rates of biallelic inactivation and genomic instability scores (GIS; ≥42) in tumors associated with six genes (*BLM*, 2; *BRIP1*, 4; *RAD51C*, 7; *RAD51B*, 2; *PALB2*, 3; and *RAD51D*, 4).[32] Although data on response to PARP inhibitors in those with germline PVs in *PALB2* are lacking in OC, studies have shown the efficacy of olaparib for germline *PALB2*-associated metastatic breast cancer (ORR, 82%),[33] and pre-clinical and clinical data suggest potential PARP inhibitor sensitivity in *PALB2*-associated prostate cancer.

These studies support our findings of heterogeneity within the group of other HR genes with respect to HRD phenotype and potential response to targeted therapies and highlight *PALB2* and *RAD51B/C/D* as promising biomarkers for targeted therapies directed to the HR pathway. Although the number of our patients with clinical HRD testing was limited, we observed concordance between WES HRD signature and clinical tests. Additionally, 50% of the patients with germline PVs in other HR genes received PARP inhibitor therapy at some point, the majority of whom had germline PVs in *BRIP1, PALB2*, and *RAD51B/C/D*. These studies and ours continue to support universal genetic testing and HRD assessments in OC.

Our findings also have implications for cancer prevention in at-risk family members. Germline PVs in *BRIP1, PALB2*, and *RAD51C/D* are established OC predisposition genes;[6] however, our study substantiates *RAD51B* as an OC predisposition gene.[34] This supports its inclusion in multigene panel testing, as few currently include it, and may warrant discussions of risk-reducing bilateral salpingo-oophorectomy in unaffected carriers to reduce OC risk. Additionally, other genes, including *ATM, BARD1*, and *NBN*, did not show high levels of biallelic inactivation, and their association with OC risk is less clear. Germline PVs in *ATM* are of particular interest given studies suggesting a moderate but consistently elevated risk of OC,[6] and recommendations for management of carriers is currently unclear.

The strengths of our study include the large number of patients with OC, including those with rare germline PVs in other HR genes, comprehensive tumor-normal sequencing, including WES in a subset of tumors with assessments of HRD using multiple methods, and treatment at a tertiary cancer center with robust clinical data and comprehensive upfront

tumor-normal sequencing, which limits bias in survival outcomes. Additionally, use of the left truncation method for the survival analyses further reduces bias as it limits the analyses to patients undergoing MSK-IMPACT close to diagnosis. The limitations of our study include the rarity of individual germline PVs in other HR genes, limiting power of comparisons, and relatively short follow-up for survival outcomes. Furthermore, clinical HRD testing data are limited, and more comprehensive and dynamic assays are needed. PARP inhibitor therapy was employed at the discretion of the treating physician and was assessed retrospectively. Additionally, we did not evaluate all known HR genes given the limitations of our germline panel and conflicting data about associations with OC; however, our study represents a comprehensive selection of the major OC predisposition genes. There may also be differences in germline findings and HRD phenotype for other rare histologies outside of high-grade serous tumor, and these should be explored in larger studies. Although we accounted for many clinical variables in our multivariable models, others may also influence outcomes, and data on treatments including PARP inhibitor therapy was limited. The cohort was also predominantly White, and racial/ethnic disparities in genetic testing are well-described.[35] Measures are needed to promote genetic testing in OC across diverse patient populations to ensure optimal treatments and promote health equity. Despite these limitations, this is an exploratory study with findings that warrant further investigation.

In conclusion, OCs with germline PVs in other HR genes represent a heterogenous group with respect to tumor HRD phenotype and possibly clinical outcomes. Although we found that *BRCA1/2*-associated OCs had favorable survival compared to WT tumors, OCs associated with germline PVs in other HR genes may have variable prognoses and response to treatment depending on the gene and tumor interactions that drive phenotype. More studies are needed to refine prognosis and determine precision therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Sharing Statement:

Data will be made available upon reasonable request through institutional processes.

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Highlights:

- Those with germline pathogenic variants (PV) in non-*BRCA1/2* homologous recombination (HR) genes were a heterogeneous group.
- There was enrichment of HR-deficient (HRD) phenotype in those with germline PVs in *PALB2* and *RAD51B/C/D* compared to *BRIP1.*
- Patients with *BRCA1/2*-associated ovarian cancer (OC) had improved survival compared to those with wildtype OC
- Survival was variable among those with germline PVs in other HR genes.
- OC associated with germline PVs in *PALB2* and *RAD51B/C/D* may have similar features to *BRCA1/2*-associated OC.

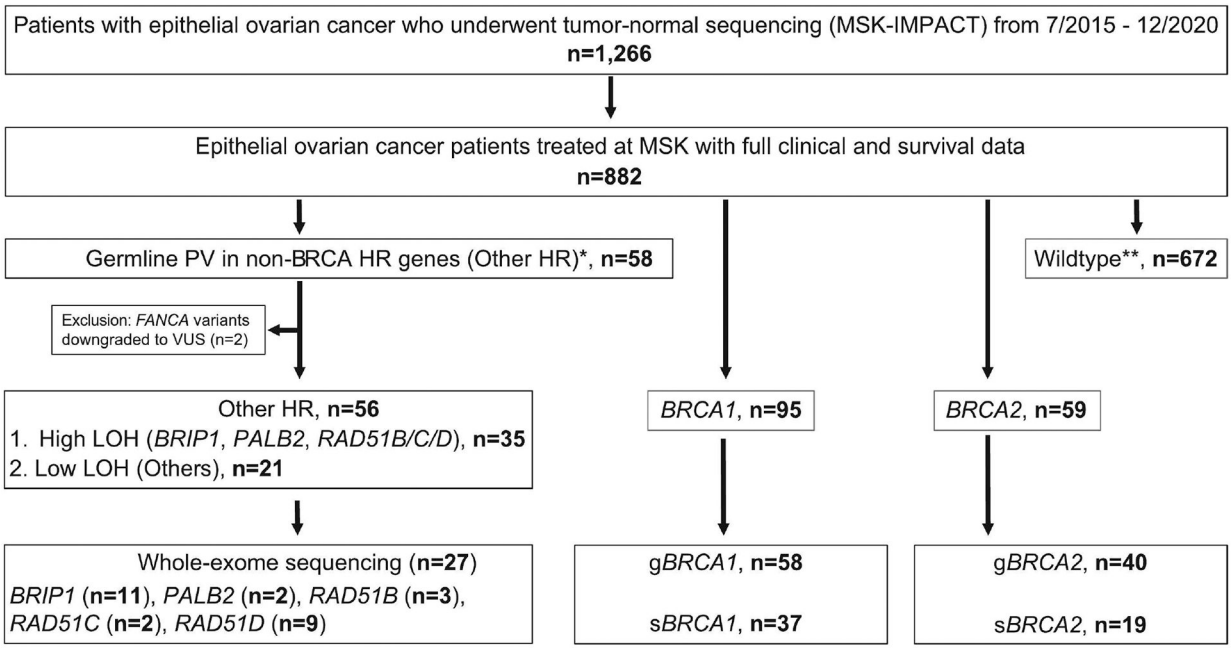

Figure 1: Patient selection and gene groups

The figure depicts the selection of all patients with epithelial ovarian cancer who underwent MSK-IMPACT from 7/2015-12/2020 at our institution and the subset treated at MSK with full clinical data as well as the breakdown by mutation status: *BRCA1, BRCA2*, Other HR, and Wildtype.

Abbreviations: PV – pathogenic variant, HR – homologous recombination, LOH – loss of heterozygosity, VUS – variant of uncertain significance, g- germline, s-somatic, MSK-IMPACT – Memorial Sloan Kettering Cancer Center – Integrated Mutation Profiling of Actionable Cancer Targets

**Other HR genes included ATM, BARD1, BRIP1, FANCA, FANCC, NBN, PALB2, RAD50, RAD51B, RAD51C*, and *RAD51D*

**WT – no germline BRCA1/2, somatic BRCA1/2 or germline PVs in other HR genes

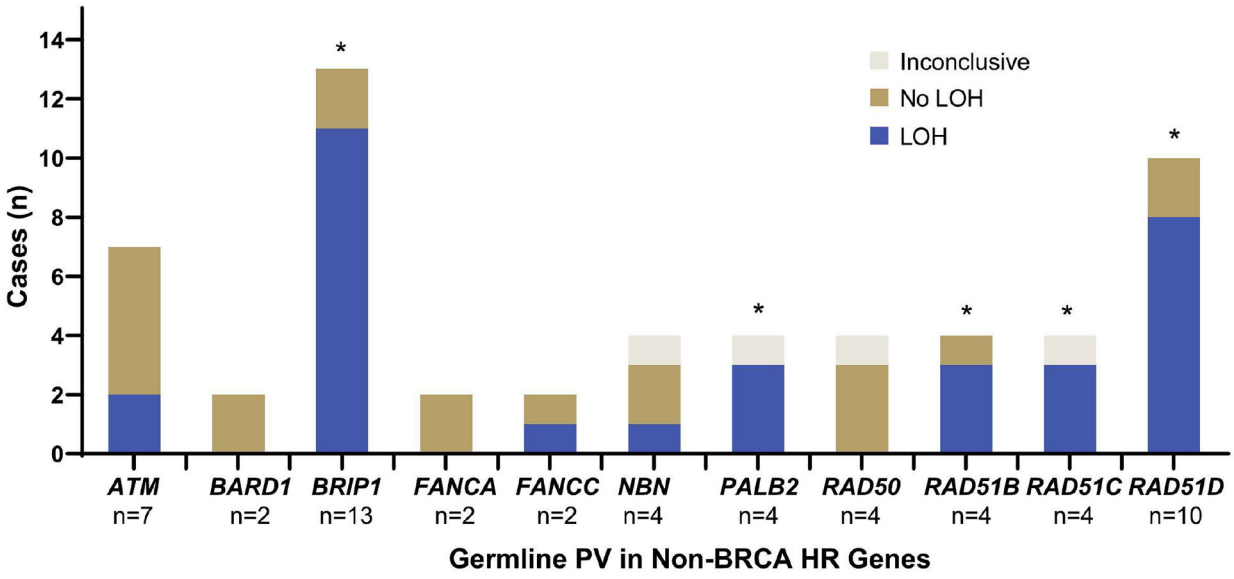

Figure 2: Loss of heterozygosity assessments for other HR genes

The figure depicts assessment of LOH and biallelic inactivation within the tumor at the germline variant identified. Two separate groups were defined based on high (≥60%) and low LOH (<60%), and WES was performed in a subset of tumors with high LOH.

Abbreviations: PV – pathogenic variant, HR – homologous recombination, LOH – loss of heterozygosity, WES – whole exome sequencing

*Denotes groups with high LOH

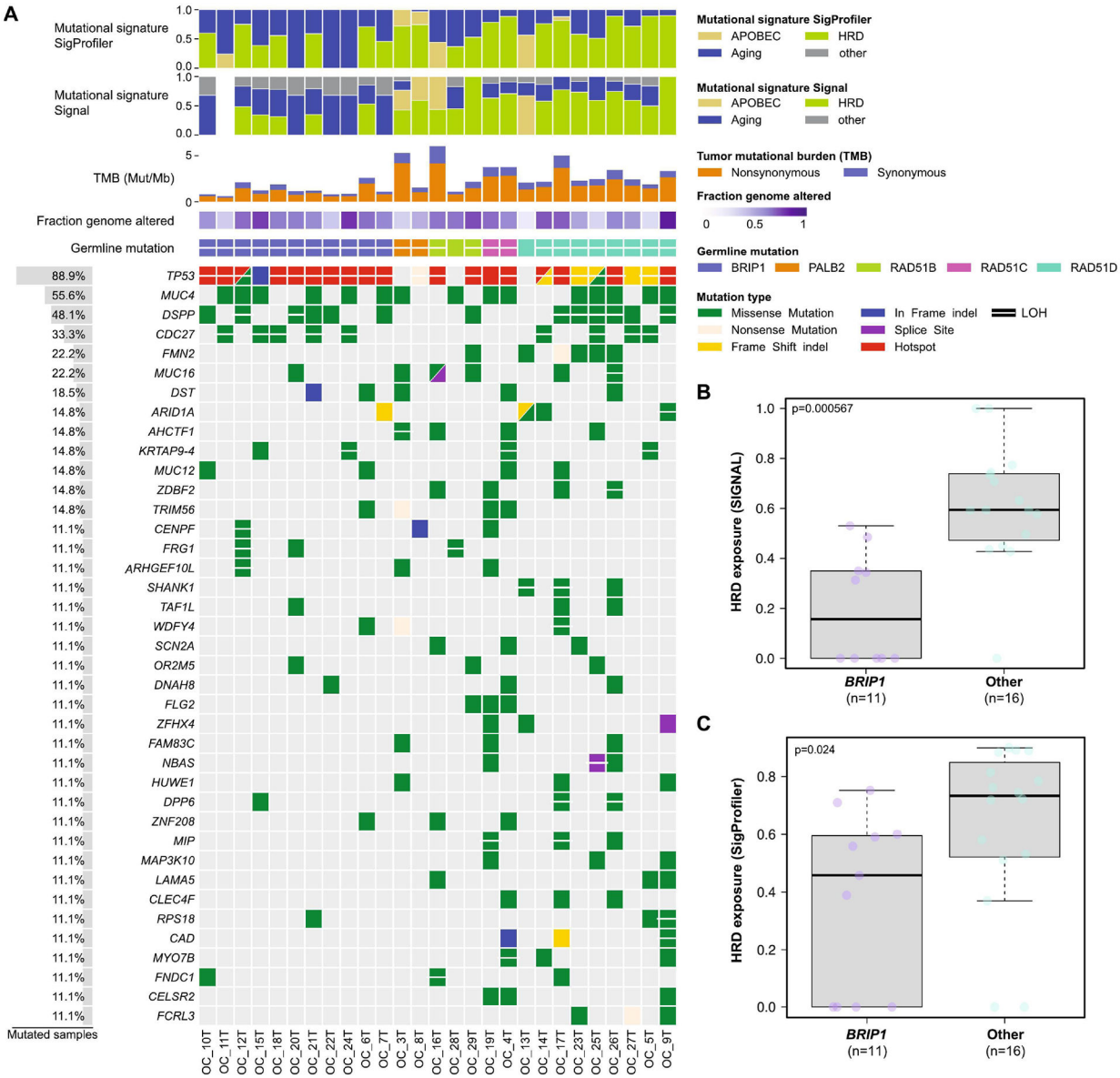

Figure 3: Whole-exome sequencing analysis in the subset of tumors associated with non-BRCA HR genes with high levels of loss of heterozygosity

The figure depicts heatmap (**A**) of mutational signatures, tumor mutational burden (TMB), fraction of genome altered (FGA), and other molecular alterations by germline pathogenic variant. Heterogeneity was observed with enrichment of homologous recombination deficiency (HRD) signatures utilizing both Signal (**B**) and SigPro (**C**) and higher TMB ($p<0.01$) in tumors with *PALB2* and *RAD51B/C/D* germline pathogenic variants compared to *BRIP1* germline pathogenic variants.

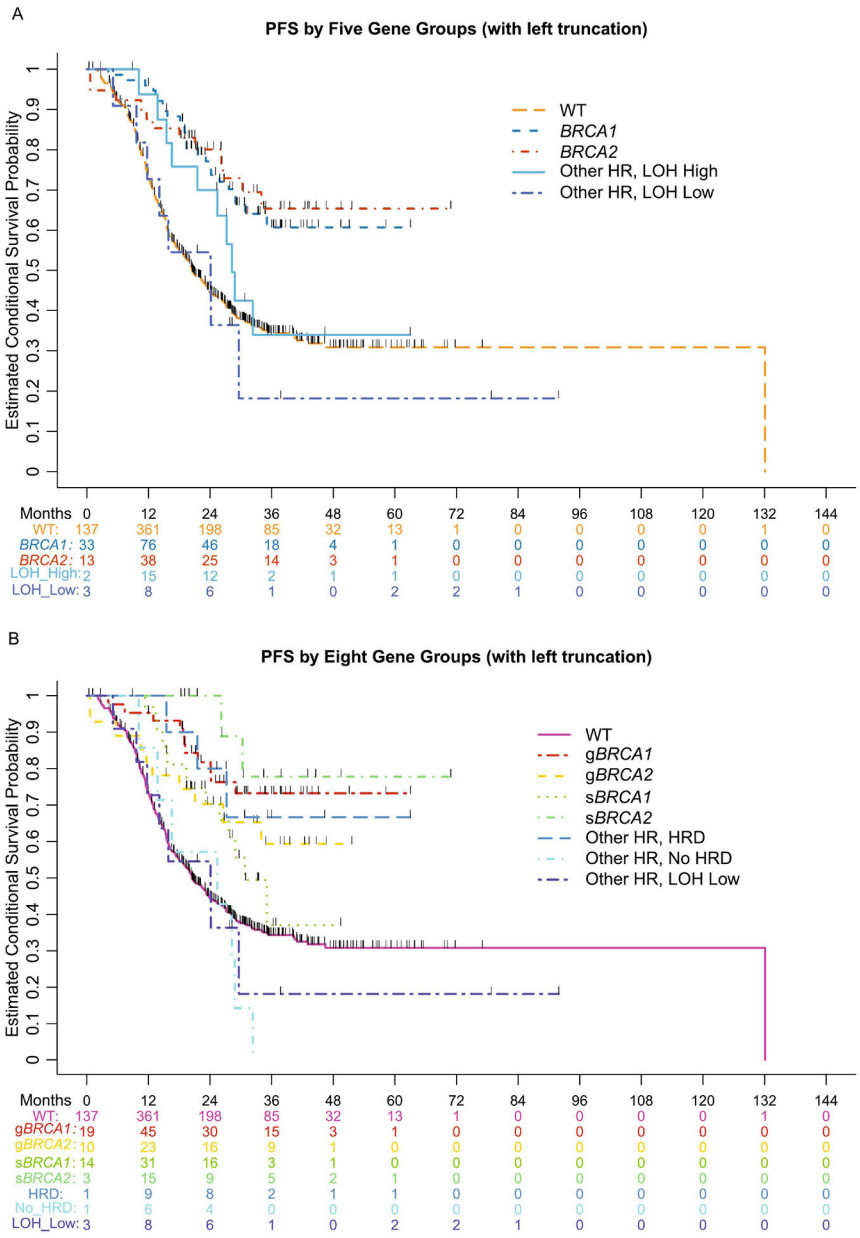

Figure 4: Progression-free survival with left truncation stratified by gene status in 5 and 8 groups

The figure depicts PFS from diagnosis by gene group, stratified into 5 groups (**A**) and 8 groups (**B**) based on *BRCA1/2* and Other HR status, incorporating LOH levels (High vs. Low), compared to WT.

Abbreviations: PFS – progression-free survival, HR – homologous recombination, LOH – loss of heterozygosity, HR – homologous recombination, HRD – homologous recombination deficiency, WT - wildtype

Table 1:

Clinical Characteristics Overall and by Gene Group

Characteristic	Overall N = 882[1]	*BRCA1* n = 95[1]	*BRCA2* n = 59[1]	Other HR n = 56[1]	Wildtype n = 672[1]	p[2]
Age at diagnosis	63 (21-93)	54 (33-85)	64 (37-83)	60 (34-88)	64 (21-93)	**<0.001**
BMI, kg/m²	25 (16-57)	25 (18-45)	26 (17-45)	26 (19-43)	25 (16-57)	0.42
Missing	22	0	0	22	0	
Obesity						0.67
No (BMI <30 kg/m²)	642(75%)	73(77%)	41(69%)	24(71%)	504(75%)	
Yes (BMI ≥30 kg/m²)	218(25%)	22(23%)	18(31%)	10(29%)	168(25%)	
Missing	22	0	0	22	0	
Initial treatment						0.47
Surgery	515(58%)	53(56%)	30(51%)	36(64%)	396(59%)	
NACT	367(42%)	42(44%)	29(49%)	20(36%)	276(41%)	
Complete gross resection						0.82
Yes	604(80%)	71(81%)	44(80%)	37(76%)	452(81%)	
No	148(20%)	17(19%)	11(20%)	12(24%)	108(19%)	
Missing	130	7	4	7	112	
Stage						**0.020**
I/II	135(15%)	7(7.4%)	6(10%)	6(11%)	116(17%)	
III	402(46%)	42(44%)	22(37%)	25(45%)	313(47%)	
IV	345(39%)	46(48%)	31(53%)	25(45%)	243(36%)	
Histology						
High-grade serous	676(77%)	91(96%)	54(92%)	44(79%)	487(72%)	
Low-grade serous	32(3.6%)	0(0%)	0(0%)	1(1.8%)	31(4.6%)	
Endometrioid	50(5.7%)	0(0%)	1(1.7%)	3(5.4%)	46(6.8%)	
Clear cell	61(6.9%)	0(0%)	1(1.7%)	7(12%)	53(7.9%)	
Carcinosarcoma	30(3.4%)	4(4.2%)	2(3.4%)	1(1.8%)	23(3.4%)	
Mucinous	14(1.6%)	0(0%)	0(0%)	0(0%)	14(2.1%)	
Other[*]	19(2.2%)	0(0%)	1(1.7%)	0(0%)	18(2.7%)	
High-grade serous histology						**<0.001**
Yes	676(77%)	91(96%)	54(92%)	44(79%)	487(72%)	
No	206(23%)	4(4.2%)	5(8.5%)	12(21%)	185(28%)	
Self-reported race						0.10
White	681(77%)	74(78%)	49(83%)	39(70%)	519(77%)	
Asian	90(10%)	13(14%)	5(8.5%)	12(21%)	60(8.9%)	
Black	46(5.2%)	5(5.3%)	3(5.1%)	1(1.8%)	37(5.5%)	
Other/unknown/missing	65(7.4%)	3(3.2%)	2(3.4%)	4(7.1%)	56(8.3%)	
Ethnicity						0.56
Hispanic	54(6.6%)	3(3.4%)	3(5.3%)	4(8.5%)	44(7.0%)	

Characteristic	**Overall N = 882[1]**	***BRCA1* n = 95[1]**	***BRCA2* n = 59[1]**	**Other HR n = 56[1]**	**Wildtype n = 672[1]**	**p[2]**
Non-Hispanic	768(93%)	86(97%)	54(95%)	43(91%)	585(93%)	
Missing	60	6	2	9	43	
Smoking						0.52
Never smoker	484(60%)	56(66%)	32(56%)	21(66%)	375(59%)	
Ever smoker	327(40%)	29(34%)	25(44%)	11(34%)	262(41%)	
Missing	71	10	2	24	35	

[1]Median (range); n(%)

[2]Kruskal-Wallis rank sum test; Fisher's exact test; Fisher's exact test for count data with simulated p value (based on 2000 replicates)

*Other: Mixed/Poorly Differentiated/Undifferentiated

Abbreviations: HR – homologous recombination, BMI – body mass index, NACT – neoadjuvant chemotherapy