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Clinical Characteristics of Ovarian Cancer Classified by *BRCA1*, *BRCA2*, and *RAD51C* Status

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We evaluated homologous recombination deficient (HRD) phenotypes in epithelial ovarian cancer (EOC) considering *BRCA1*, *BRCA2*, and *RAD51C* in a large well-annotated patient set. We evaluated EOC patients for germline deleterious mutations (n = 899), somatic mutations (n = 279) and epigenetic alterations (n = 482) in these genes using NGS and genome-wide methylation arrays. Deleterious germline mutations were identified in 32 (3.6%) patients for *BRCA1*, in 28 (3.1%) for *BRCA2* and in 26 (2.9%) for *RAD51C*. Ten somatically sequenced patients had deleterious alterations, six (2.1%) in *BRCA1* and four (1.4%) in *BRCA2*. Fifty two patients (10.8%) had methylated *BRCA1* or *RAD51C*. HRD patients with germline or somatic alterations in any gene were more likely to be high grade serous, have an earlier diagnosis age and have ovarian and/or breast cancer family history. The HRD phenotype was most common in high grade serous EOC. Identification of EOC patients with an HRD phenotype may help tailor specific therapies.

Ovarian cancer represents the fifth most common cause of cancer mortality in women due in part to the advanced stage at which patients typically present, with an estimated 14,030 deaths and 22,240 cases in 2013 in the United States[1]. The most common ovarian cancer is invasive epithelial ovarian cancer, with five common histological subtypes: high grade serous (70%), endometrioid (10%), clear cell (10%), low grade serous (5%), and mucinous (3%). A family history of breast or ovarian cancer in first degree relatives increases the risk for ovarian cancer[2]; family-based studies have revealed high- and moderate-penetrance genes including *BRCA1*[3], *BRCA2*[4], DNA mismatch repair genes[5], *RAD51C*[6,7], *RAD51D*[8] and *BRIP1*[6], and case-control studies have identified eleven common variants associating with modestly increased risks[9–15]. Estimates of the contribution of germline *BRCA1* and *BRCA2* mutations to EOC vary widely from 5% to 20%[16]; somatic mutations in *BRCA1* and *BRCA2* occur less frequently (in 2%–8% patients)[17,18] and, as has *BRCA1* methylation[19–22] have been reported in ovarian cancer patients with no family history[23].

Ovarian cancer patients who are *BRCA1* and *BRCA2* germline mutation carriers have been reported to have an improved outcome compared to non-carriers and the mechanism underlying this benefit has been hypothesized as a high response rate to platinum agents, particularly among patients with high grade serous histology[24–27]. This is because patients with germline *BRCA1* or *BRCA2* mutations have an impaired ability to repair double stranded DNA breaks through homologous recombination[28]. More recently, favorable responses to poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitors have been noted in ovarian cancer patients carrying deleterious germline *BRCA1* and *BRCA2* mutations[29]. However, some ovarian cancer patients without germline *BRCA1* and *BRCA2* mutations also respond to PARP inhibitors[30], suggesting that broader dysfunction of homologous recombination than *BRCA1* and *BRCA2*, a homologous recombination deficient (HRD) phenotype may

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be important. HRD may be the result of deleterious germline mutation in other homologous recombination genes such as *RAD51B*, *RAD51C*, or *RAD51*D or somatic mutation or silencing of the same homologous recombination genes[31,32]

We evaluated the prevalence of HRD in a large EOC case series and correlated this with clinical outcomes. HRD was determined by sequencing *BRCA1*, *BRCA2* and *RAD51C* in germline and tumor DNA and assessing DNA methylation of cytosines at CpG sites surrounding these genes.

Results

Table 1 shows the characteristics of patients and Figure 1 shows the number of samples with each data type, after QC exclusions, included in this analysis.

Frequency of methylation, germline and somatic mutations. Of the 899 germline-tested patients, deleterious mutations were seen in 83 (9%): 32 (3.5%) in *BRCA1*, 28 in *BRCA2* (3%), and 26 in *RAD51C* (2.9%; Table 2). Three cases carried mutations in more than one gene: two patients carried deleterious mutations in *BRCA1* and *RAD51C*, and one carried deleterious mutations in *BRCA2* and *RAD51C*. For the 619 HGS cases, deleterious mutations were seen in 69 (11%), 27 (4%) in *BRCA1*, 24 (4%) in *BRCA2* and 19 (3%) in *RAD51C*. One HGS patient carried a mutation in both *BRCA2* and
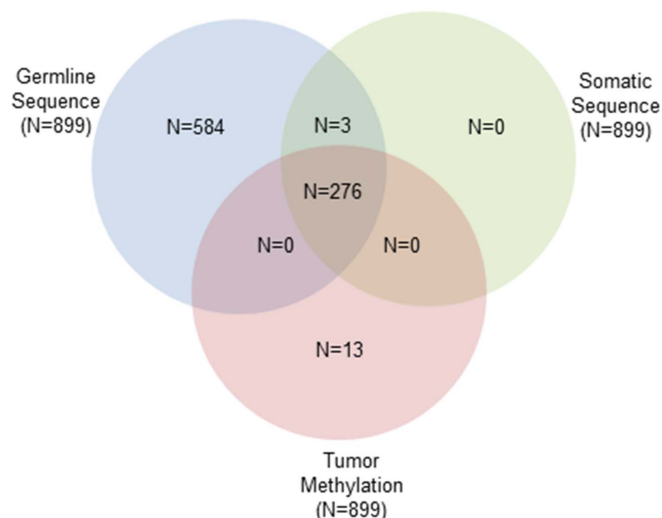

Figure 1 | Venn diagram representing data availability for N = 1063 invasive EOC patients.

RAD51C. Of the six Ashkenazi Jewish patients, one carried a germline *BRCA1* mutation (C61G) and none carried either somatic mutations or methylation. Table S1 and S2 provides a complete mutation listing, after exclusions (see Methods section). Another 187 women carried germline missense mutations, none of which had evidence for pathogenicity in the key clinical databases, 65 of these alterations were in *BRCA1*, 102 in *BRCA2* and 32 in *RAD51C*. Twelve patients had a missense mutation in multiple genes. These variants were considered variants of unknown significance (VUS). In the patients with deleterious mutations, 13 patients also had a missense VUS that was identified. The remaining 629 patients had no deleterious or missense VUS mutations in *BRCA1*, *BRCA2* or *RAD51C*.

Two hundred and seventy nine patients with available tumor tissue were screened for somatic mutations. Ten tumors (4%) were found to have deleterious somatic mutations, six in *BRCA1*, and four in *BRCA2* (Table 2). Table S2 provides a complete listing for the somatic mutations. Missense VUS were also detected with one in *BRCA1* and one in *BRCA2*. Two hundred and sixty seven patients had no deleterious or missense VUS mutations detected. No deleterious somatic variations were identified in *RAD51C*.

Forty five of 482 tumors (9%) exhibited a methylated BRCA1 phenotype, and seven tumors (1%) showed RAD51C methylation (Table 2). *BRCA1* methylation was not detected in any individual carrying a germline *BRCA1* deleterious mutation; similarly, *RAD51C* methylation was exclusive of germline *RAD51C* mutation carrier status. However, there were two patients with a mutation and methylation of another gene; one patient had a somatic mutation in *BRCA2* and *BRCA1* methylation and another carried a germline RAD51C mutation and had methylated *BRCA1*.

Clinical and pathological analyses. In combination, 143 patients exhibited a HRD phenotype (82 due to germline mutation only, nine due to somatic mutation only, 50 due to tumor methylation only, one due to germline mutation and methylation, and one additional due to somatic mutation and methylation, Table 2). A total of 79 HRD patients showed alterations in *BRCA1*, 30 in *BRCA2*, 29 in *RAD51C* and five patients showed alterations in more than one gene.

We examined the clinical features of HRD compared to patients with no abnormality detected (NAD) for those with both germline and somatic data (Tables 3, S3). HRD patients had younger age at diagnosis (P = 0.0001) and were more likely to be high grade serous (118/264, P = 0.0004) and have a family history of breast or ovarian

Table 1 | Clinical characteristics of epithelial ovarian cancer patients (N = 1063)

	N (%)
Mean age at diagnosis, years (SD)	62.4 (11.7)
Histology	
High-grade serous	735 (70%)
High grade endometrioid	73 (7%)
Low grade endometrioid	67 (6%)
Clear Cell	69 (7%)
Low-grade serous	34 (3%)
Mucinous	29 (3%)
Other/Unknown	56
FIGO Stage	
I	158 (15%)
II	62 (6%)
III	637 (61%)
IV	194 (19%)
Unknown	12
Debulking category	
Optimal	848 (85%)
Sub-optimal	150 (15%)
Unknown	65
Grade	
Low grade	143 (14%)
High grade	896 (86%)
Unknown	24
Menopause status	
Pre-menopausal/peri-menopausal	169 (20%)
Post-menopausal	672 (80%)
Unknown	222
Ascites present at surgery	
Yes	524 (63%)
No	307 (37%)
Unknown	232
Ashkenazi ancestry	
Yes	6 (1%)
No	498 (99%)
Unknown	559
Median survival, years (range)	2.9 (0.01–10)
Median follow-up time among living, years (range)	4.5 (0.01–10)
Deaths	638 (60%)

N (%), unless otherwise specified.

Table 2 | Summary of germline mutations, somatic mutations, and methylation of *BRCA1*, *BRCA2* and *RAD51C*

	N Tested	Gene, n			
		BRCA1	*BRCA2*	*RAD51C*	Combined HRD
Germline deleterious mutation	899	32	28	26	83
Somatic deleterious mutation	279	6	4	0	10
Methylated	482	45	-	7	52
No deleterious mutation, not methylated	NA	237	261	263	NA
No deleterious germline mutation, unknown somatic mutation and/or tumor methylation status	NA	592	606	604	NA
Not methylated, germline and somatic mutation status unknown	NA	151	164	163	NA
Total		1063	1063	1063	143

Deleterious mutations were frame shift insertion/deletion, splice site, rare missense supported by multiple methods to be damaging; NA, not applicable; one case carried germline deleterious mutations for both *BRCA2* and *RAD51C*, and two patients carried germline deleterious mutations for *BRCA1* and *RAD51C*, thus the combined number of germline deleterious mutation carriers is less than the sum of mutation carriers for each gene; one case carried a RAD51C germline deleterious mutation and methylated for BRCA1 and another case carried a BRCA2 somatic deleterious mutation and methylated for BRCA1, thus the total number of patients with a HRD phenotype is less than the sum of germline mutation carriers and patients with a somatically mutated or methylated gene.

cancer (P = 0.002) than patients with NAD. Interestingly, patients with high grade (10/27) endometrioid EOC were HRD, compared to 2/23 patients with low grade endometrioid EOC. One of the most common pathology challenges is whether high grade endometrioid are in fact high grade serous EOC[33]. Other covariates (debulking, peritoneal cytology, the presence of ascites and smoking and alcohol use history) were not significantly different between the two groups. We also examined clinical features within the HRD patients and found only family history of breast or ovarian cancer was more common in mutated than methylated patients (P = 0.0001, data not shown).

Survival analyses were carried out on those patients with HRD and those with NAD for which all data (germline, somatic mutation and methylation) were available (n = 356). Forty percent (143/356) of all the patients were HRD, while 44.7% (118/264) of HGS patients were HRD. Overall survival for all patients was associated with the HRD phenotype (adjusted HR 0.73, 95% CI 0.54–0.98, P = 0.04, Table 4), but not within each of the defective classes, genes that were mutated at the germline or somatic level or were somatically methylated (respectively, adjusted HR 0.81, 95% CI 0.57–1.15; adjusted HR 0.56, 95% CI 0.20–1.54, adjusted HR 0.65, 95% CI 0.42–1.02, P = 0.18, Table 4), or when outcomes were examined by germline mutation carrier status (P = 0.09, Table 4). The hazard ratios data are

similar to those previously reported in a large germline study[26]. Results were similar whether VUS were included in the NAD category or excluded (data not shown). Analysis of time to recurrence showed no association with any of the HRD phenotypes (Table S3). Survival analyses were also conducted restricted to high grade serous (n = 618 for germline carrier analysis, 264 for HRD analysis) and showed an overall survival differences between HRD and NAD (adjusted HR 0.70, 95% CI 0.51–0.97, P = 0.03, Table 4, Figure 2), but not between germline mutation carriers and-non carriers (adjusted HR 1.07, 95% CI 0.65–1.76; adjusted HR 0.76, 95% CI 0.45–1.30, adjusted HR 0.66, 95% CI 0.37–1.18, P = 0.40, Table 4, Figure 2). Survival at 5 years was similar (adjusted HR 0.68 95% CI 0.48, 0.97, P = 0.04, data not shown). Analysis of time to recurrence was null (Table S4, Figure S3). Finally, we examined HRD phenotypes by debulking status in germline mutation carriers, as Alsop et al[27] reported that improved survival was not evident in patients that were sub-optimally debulked. However, we observed no difference in germline mutation carriers stratified by debulking status (data not shown).

Discussion

Using a large homogeneous collection of ovarian cancer patients with clinical follow up unselected for family history, we evaluated

Table 3 | Comparison of the clinical characteristics of HRD to NAD patients

	HRD (n = 143)	NAD (n = 213)	P value
Age at Diagnosis, years			0.0001
Mean (SD)	58.4 (10.6)	63.1 (11.5)	
Histology			0.0004
High grade serous	118 (45%)	146 (55%)	
High grade endometrioid	10 (37%)	17 (63%)	
Low grade endometrioid	2 (9%)	21 (91%)	
Clear Cell	5 (28%)	13 (72%)	
Low grade Serous	2 (15%)	11 (85%)	
Mucinous	0 (0%)	5 (100%)	
Other/Unknown	6 (100%)	0 (0%)	
Grade			<0.0001
High	135 (44%)	174 (66%)	
Low	5 (11%)	39 (89%)	
Unknown	3 (100%)	0 (0%)	
First Degree Family History of Breast or Ovarian Cancer			0.0020
No	104 (36%)	183 (64%)	
Yes	39 (57%)	30 (43%)	
First Degree Family History of Ovarian Cancer			0.0344
No	132 (39%)	207 (61%)	
Yes	11 (65%)	6 (35%)	

Only patients with known HRD status (germline mutation, somatic mutation and somatic methylation) available were included; VUS carriers are included in NAD patient group; stage, debulking, presence of ascites, peritoneal cytology, smoking history, enrollment year, alcohol use were not significantly different between HRD and NAD patients.

the clinical and pathological characteristics associated with a variety of HRD phenotypes. Deleterious germline mutations in *BRCA1*, *BRCA2* or *RAD51C* were seen 9% of the subjects, *BRCA1* or *BRCA2* in 6.7% which is within the ranges of those previously reported (5.8%-13.2%)[16]. Somatic mutations were less common (3.6%), but 11% of the tumors analyzed exhibited BRCA1 or RAD51C methylation, comparable to the prevalence observed in 319 high grade serous ovarian cancers analyzed by the Cancer Genome Atlas[19]. In total, 143 of the patients had an HRD phenotype. A common phenotype has been reported for sporadic cancers that share the characteristics of tumors associated with germline *BRCA1* and *BRCA2* mutations[34,35], and includes *RAD51C* in which germline mutations have been identified in patients of familial breast and ovarian cancer[6,36,37], as has methylation[38,39]. Additionally, Abkevich et al[40] report an LOH-based score for HRD which included promoter methylation of *RAD51C*. Here, we analyzed patients regardless of family history, included germline as well as tumor testing for more comprehensive analysis and defined patients as HRD if any deleterious alterations in *BRCA1*, *BRCA2* and *RAD51C* genes were found.

An HRD phenotype was seen in 45% of high grade serous EOC (69% of patients had HGS EOC), with a smaller proportion observed in low grade serous and the other histologies. This is consistent with observations of others[19,26,27]. While we did observe a survival advantage for those patients with an HRD phenotype, analysis did not reveal any overall survival differences between *BRCA1* or *BRCA2* mutation carriers and non-carriers, unlike prior studies. This was unexpected given the literature suggesting longer progression free survival or overall survival for EOC in mutation carriers; however, this advantage appears to be short term. Most studies were out to 60 months only and McLaughlin et al[41] showed that an early (up to five years) survival benefit was not evident beyond eight years[41]. Similarly we do not show a long term survival advantage, thus follow up time may explain the apparent discordance. It may also be that the number of germline *BRCA1* and *BRCA2* mutations carriers was too small and thus underpowered. Additionally, while care was taken to classify the mutations according to the best available evidence, the power of our analysis will have been reduced by any misclassification of HRD. While the TCGA concluded that methylated patients more closely match survival in wild type BRCA patients, we observed that methylated cases may have a survival benefit similar to that of the germline mutation patients. All categories, while not reaching statistical significance, showed a trend towards a survival benefit; had methylation not been thus, then combining with mutation positive patients would have biased the testing procedure towards the null. Biologically, *BRCA1* inactivated by methylation would result in a similar defect as a deleteriously mutated allele. Our study involves patients at one site, and all data were similarly processed while the TCGA study involves samples from multiple centers with multiple data sets, which may also account for the differences.

The high frequency of HRD defects in high grade serous EOC suggest screening for this phenotype should be considered in clinical care, particularly for new therapies, such as the PARP inhibitors that may be most efficacious within this phenotype.

Strengths of this report include detailed methylation phenotyping of a subset of patients and sequencing of tumor DNA. Limitations include lack of methylation and somatic mutation data on up to 67% of patients, not confirming the detected mutations in every patient and inclusion of only three of more than ten genes now considered to contribute to the HRD phenotype[42]. It is also critical to note that most of these patients were treated with the current standard of care at time of diagnosis (carboplatin and/or taxane); upcoming studies will focus on those treated with PARP inhibitors. Other future work includes sequencing of additional DNA repair genes in order to more comprehensively identify those patients with defective homologous recombination pathway abnormalities.
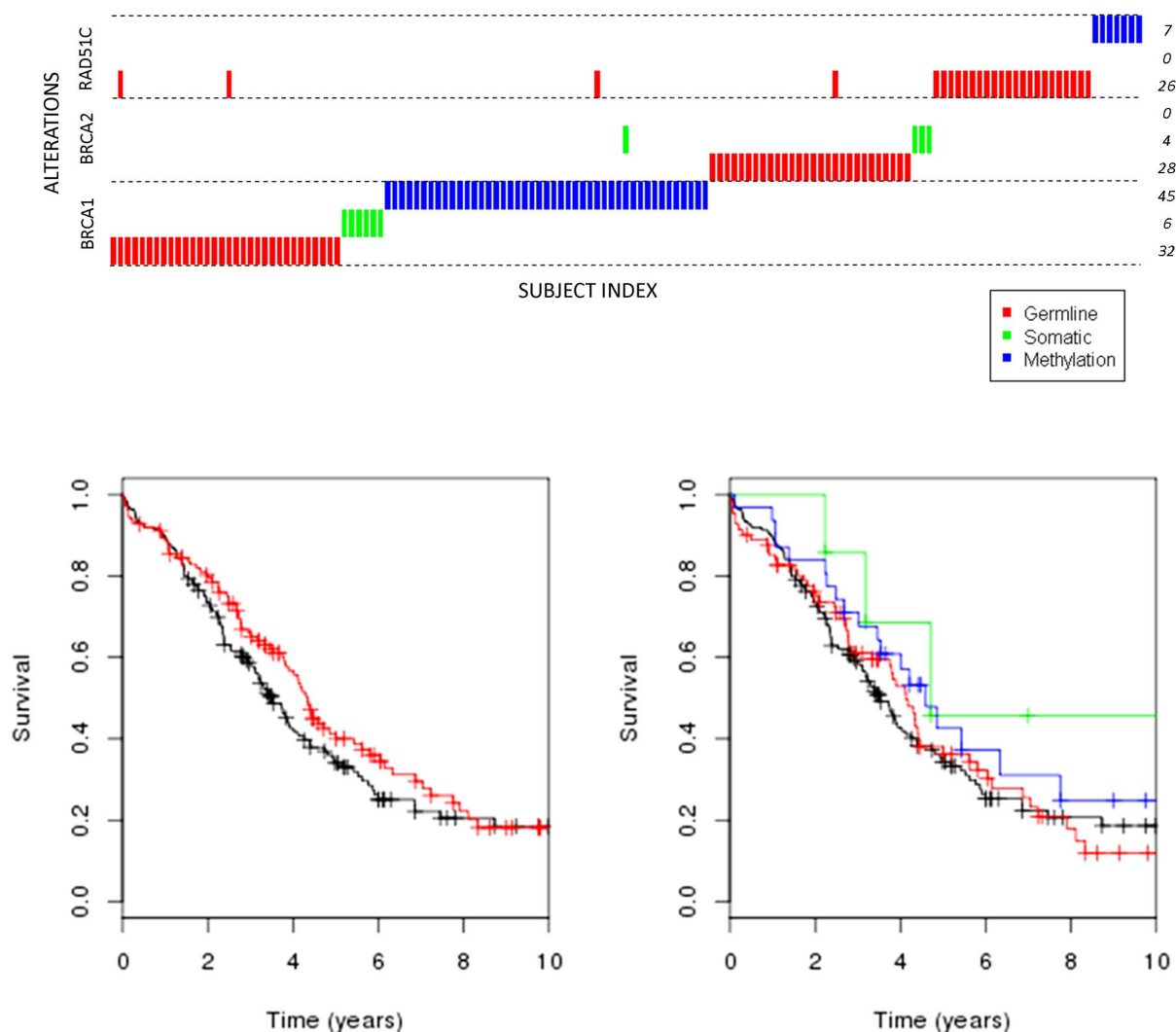
Table 4 | Analysis of overall survival by HRD phenotypes

	All patients				Covariate-adjusted		High grade serous				Covariate adjusted	
	N	N (%) deaths	HR (95% CI)	P value	HR (95% CI)	P value	N	N (%) deaths	HR (95% CI)	P value	HR (95% CI)	P value
By HRD												
NAD	213	131 (62%)	ref		ref		146	100 (68%)	ref		ref	
HRD	143	84 (59%)	0.86 (0.65,1.13)	0.27	0.73 (0.54,0.98)	0.04	118	74 (63%)	0.74 (0.55,1.00)	0.05	0.70 (0.51,0.97)	0.03
By Type of Alteration												
None	213	131 (62%)	ref		ref		146	100 (68%)	ref		ref	
Germline mutation	82	48 (59%)	0.89 (0.64,1.24)		0.81 (0.57,1.15)		68	44 (65%)	0.81 (0.56,1.15)		0.80 (0.56,1.16)	
Somatic mutation	9	4 (44%)	0.67 (0.25,1.82)		0.56 (0.20,1.54)		7	3 (43%)	0.47 (0.15,1.48)		0.42 (0.13,1.33)	
Methylation	50	32 (64%)	0.88 (0.60,1.30)	0.75	0.65 (0.42,1.02)	0.18	41	27 (66%)	0.74 (0.49,1.14)	0.27	0.63 (0.39,1.02)	0.13
By Germline Mutation												
Non-carrier	816	466 (57%)	ref		ref		550	367 (67%)	ref		ref	
BRCA1	30	19 (63%)	0.99 (0.62,1.56)		1.16 (0.72,1.85)		27	17 (63%)	0.75 (0.46,1.22)		1.07 (0.65,1.76)	
BRCA2	27	14 (52%)	0.79 (0.46,1.34)		0.64 (0.38,1.10)		23	14 (61%)	0.77 (0.45,1.32)		0.76 (0.45,1.30)	
RAD51C	23	12 (52%)	0.63 (0.36,1.12)	0.37	0.58 (0.33,1.04)	0.09	18	12 (67%)	0.62 (0.35,1.10)	0.20	0.66 (0.37,1.18)	0.40

Women with VUS in any of the three genes were included in the NAD and non-carrier groups; results did not differ when these individuals were excluded from the analyses; adjusted results were adjusted for age at diagnosis, debulking status, stage, grade (low/high), ascites present at surgery, and menopausal status including categories for patients with missing data.

Figure 2 | Overall survival for high grade serous EOC by HRD and type of alteration. (A). Red: HRD; Black: NAD. (B). Above is a depiction of samples with type of alteration in each gene; below are the Kaplan Meier curves. Red: germline deleterious mutation; Green: somatic deleterious mutation; Blue: somatic methylation; Black: no germline mutation, somatic mutation or methylation.

Methods

Patient population and biospecimens. Women with EOC (n = 1063) provided written informed consent between 1992 and 2011 using IRB approved processes at the Mayo Clinic in Rochester, MN. Use of patient derived samples in this study was also approved and the work described was carried out in accordance with these approved guidelines. Eligible participants were women aged 20 years or older with diagnosis of pathologically confirmed invasive primary epithelial ovarian, fallopian tube or primary peritoneal cancer. Patients were followed for vital status using electronic medical records and linkage to vital statistics sources; the median time of follow up was 4.5 years, with a range of 0.1–10 years. Most patients were treated with a platinum agent (carboplatin or cisplatin) and taxane, the standard of care at time of diagnosis. Diagnosis (histology and grade) were reviewed by a gynecologic pathologist. Peripheral blood (n = 899) was used a source of germline DNA[43]. Pre-chemotherapy tumor tissue (up to n = 482) was snap frozen immediately following surgery and stored at −80°C; tumor DNA was prepared from cryostat sections using Qiagen PureGene chemistry (Qiagen Inc, Valencia, CA, USA) and quantitated using Trinean (Trinean, Gentbrugge, Belgium). Tumor RNA was isolated from fresh frozen samples using the Qiagen RNEasy protocol (Qiagen Inc, Valencia, CA, USA) and quantitated using a Nanodrop Spectrophotomer (Agilent Technologies, Santa Clara, CA, USA). Somatic methylation analyses preceded the mutation screening, thus samples with insufficient DNA for both analyses were not sequenced.

DNA sequencing and bioinformatics. Germline DNA (n = 899, 1.25 μl DNA at a concentration 75 ng/μl) and tumor DNA (n = 279) were sequenced at the University of Cambridge, Cambridge, UK at *BRCA1*, *BRCA2* and *RAD51C*. The procedure entailed design of PCR primers, testing and optimization using Fluidigm Access Array (Fluidigm Corp., San Francisco, CA, USA). Briefly, the method used a 4-primer amplicon-tagging scheme. Tagged, target-specific primer pairs were combined with sample-specific primer pairs that contained barcoding sequences and the adaptor

sequences used by the Illumina sequencing system. Using sample-specific barcodes, all PCR products generated in the 48.48 Access Array Integrated Fluidic Circuit were unique and pooled together to run in a single sequencing experiment. Sequencing reactions were carried out on an Illumina GAIIX next-generation sequencer (Illumina, Inc., San Diego, CA, USA).

GENOME_GPS 1.0 was used for analysis of DNA sequence data including read alignment, variant detection, and annotation (Figure S1). Reads were aligned to genome build hg19 using Novoalign and realignment, recalibration used the Genome Analysis Toolkit (GATK) v.1.6.7[44]. The percent of reads mapped on target was used to exclude seven germline samples with <80% of amplicons covered at 20× and twenty one tumor samples with <80% of the amplicons covered at 20×. To avoid false-negative deleterious carrier status assignments for the somatic mutation data, non-carrier patients were excluded if either germline or tumor sequencing data exhibited <80% on-target sequence coverage at 20× or greater [mean coverage, 1st and 3rd quartiles: *RAD51C* 138.76 (121.68, 150.94); *BRCA1* 164.81 (142.59, 179.47); *BRCA2* 157.65 (133.68, 174.13); all three genes, 158.20 (136.06, 173.04)].

Germline variants were called through GATK Unified Genotyper. Germline filtering used genotype quality greater than 20 and read depth greater than 20. Somatic single nucleotide variants were called using SomaticSniper[45], whereas insertions and deletions were called by GATK Somatic Indel Detector[44]. Somatic variants were considered with a depth of at least 20× coverage for the germline and tumor DNA at the given site as well as a quality score ≥ 20 and excluded if close to indel calls, aligned to multiple positions and/or occurred in a known repeat region. Indel calls required ≥ 10 supporting tumor sequencing reads, and no reads supporting the indel in the germline.

Each variant was annotated using the Targeted RE-sequencing Annotation Tool (TREAT)[46]. Missense coding variants were functionally annotated by snpEFF (http://snpeff.sourceforge.net/) and PolyPhen-2 to predict biological effects. Somatic variants were considered deleterious if they were stop-gains (nonsense) or occurred at splice sites junctions, along with frameshifts and insertion/deletions as deleterious. In

addition, missense mutations were hand-curated using LOVD (www.LOVD.nl), IARC (www.iarc.fr), HGMD (www.hgmd.cf.ac.uk), and Alamut (http://www.interactive-biosoftware.com/software/alamut/features) databases. Variants for which deleteriousness could not be determined with confidence, or which were known polymorphisms, were considered VUS.

DNA methylation. Tumor methylation status for 482 patients was examined using the Illumina Infinium HumanMethylation450 BeadChip as previously described[47]. Positive bisulfite modified (BSM) controls (SssI treated DNA, CpGenome Universal Methylated DNA, Millipore, Billerica, MA, USA, and placental DNA), negative BSM controls (unmodified SssI treated DNA and WGA DNA), and a CEPH DNA (Coriell Institute), were included in each 96 well plate. Validation of nine CpG loci, including one at BRCA1 (cg25288140), was accomplished by Pyrosequencing, as previously described[47] and suggested that BeadChip data were satisfactory; Pearson correlation of methylation values between BeadChip and Pyrosequencing assays ranged from 0.57 to 0.96.

To dichotomize tumor methylation status for each gene, we used expression array data from 171 patients to identify CpG probes which negatively correlated with expression. Expression data were generated using 750 ng of high quality total RNA and Agilent Whole Human Genome 4 × 44K Expression Arrays (using a mixed reference of 107 tumors) as previously described[47]. We estimated pairwise Spearman correlations between gene expression and CpG methylation probes within 20 kb of each gene to identify CpG probes most associated with expression. For each probe, we confirmed that each CpG probe was close to known promoter and active regulatory elements defined by ENCODE project using the UCSC Genome Browser (Figure S2A). Thresholds for dichotomization of methylation status were defined based upon the median methylation value of the selected probe set. For example, Figure S2B plots BRCA1 expression versus the median of N = 21 BRCA1 CpG probes; based on this, patients with a BRCA1 CpG methylation > 0.15 were considered BRCA1 methylated. A similar process was used for six CpG probes in RAD51C; however, no BRCA2 CpG was clearly associated with altered gene expression, thus BRCA2 was not part of methylation analyses.

Association analysis. HRD patients were defined as those with deleterious germline mutations, deleterious somatic mutations or methylation of BRCA1, BRCA2, or RAD51C. Clinical characteristics of HRD and patients with NAD were compared using Fisher exact tests. Survival analysis with HRD status was conducted using multivariable Cox proportional hazards modeling and, for adjusted analyses, included age, stage, debulking, menopausal status, grade, and ascites, estimating hazard ratios (HRs) and 95% confidence intervals (CIs). Time at risk was defined from the date of diagnosis to date of death allowing for left truncation with right censoring at last date of follow up to 10 years. In addition to overall survival, we considered time-to-recurrence or death from any cause. We analyzed survival using the HRD phenotype defined by germline status only and also by combined germline, somatic, and methylation status (using the subset with complete data). Analyses were for all patients and among patients with high grade serous EOC and were conducted both with VUS considered to be non-carriers or excluded. Each analysis used all available data (thus sample sizes vary) and was implemented in SAS, with corresponding Kaplan-Meier curves generated in R.

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Author contributions

E.L.G. and P.P.D.P. designed the study. J.M.C. coordinated the analyses and prepared the manuscript. All authors read and approved the final version of the manuscript. M.C.L., N.B.L., C.W., B.L.F., E.M.D. and J.D. performed statistical and informatics analyses. G.C., H.H. and G.E.K. provided the gene expression data. H.S., P.H. and P.P.D.P. performed the next generation sequencing. J.M.C. performed the methylation analysis. Sample provision and management were coordinated by K.K. Clinical information was provided by B.W., N.M.L., M.W. and M.B. Technical support and advice were provided by M. C., J.C., M.B. and J.-B.F.

Additional information

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