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ORIGINAL REPORT

Contribution of Germline Mutations in the *RAD51B*, *RAD51C*, and *RAD51D* Genes to Ovarian Cancer in the Population

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose
The aim of this study was to estimate the contribution of deleterious mutations in the *RAD51B*, *RAD51C*, and *RAD51D* genes to invasive epithelial ovarian cancer (EOC) in the population and in a screening trial of individuals at high risk of ovarian cancer.

Patients and Methods
The coding sequence and splice site boundaries of the three *RAD51* genes were sequenced and analyzed in germline DNA from a case-control study of 3,429 patients with invasive EOC and 2,772 controls as well as in 2,000 unaffected women who were *BRCA1/BRCA2* negative from the United Kingdom Familial Ovarian Cancer Screening Study (UK_FOCSS) after quality-control analysis.

Results
In the case-control study, we identified predicted deleterious mutations in 28 EOC cases (0.82%) compared with three controls (0.11%; $P < .001$). Mutations in EOC cases were more frequent in *RAD51C* (14 occurrences, 0.41%) and *RAD51D* (12 occurrences, 0.35%) than in *RAD51B* (two occurrences, 0.06%). *RAD51C* mutations were associated with an odds ratio of 5.2 (95% CI, 1.1 to 24; $P = .035$), and *RAD51D* mutations conferred an odds ratio of 12 (95% CI, 1.5 to 90; $P = .019$). We identified 13 *RAD51* mutations (0.65%) in unaffected UK_FOCSS participants (*RAD51C*, n = 7; *RAD51D* , n = 5; and *RAD51B*, n = 1), which was a significantly greater rate than in controls ($P < .001$); furthermore, *RAD51* mutation carriers were more likely than noncarriers to have a family history of ovarian cancer ($P < .001$).

Conclusion
These results confirm that *RAD51C* and *RAD51D* are moderate ovarian cancer susceptibility genes and suggest that they confer levels of risk of EOC that may warrant their use alongside *BRCA1* and *BRCA2* in routine clinical genetic testing.

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INTRODUCTION

Epithelial ovarian cancer (EOC) has a significant heritable component. A woman with a single first-degree relative diagnosed with ovarian cancer has a three-fold increased risk of the disease.[1,2] Twin studies suggest that most of the familial clustering results from inherited genetic factors.[3] High-penetrance mutations in *BRCA1* and *BRCA2* are associated with the majority of breast-ovarian cancer syndrome occurrences.[4-6] The cumulative estimated risks of ovarian cancer averaged across all possible polygenic risk modifiers by age 70 years are 36% in *BRCA1* carriers and 12% in *BRCA2* carriers.[7]

Other ovarian cancer susceptibility genes include the mismatch repair genes *MSH6*, *MSH2*, and *MLH1*,[8] which also are associated with colorectal and endometrial cancers. Several common low-penetrance susceptibility alleles conferring relative risks (RRs) of less than 1.5-fold have been found using genome-wide association studies.[9-17] The known high-risk susceptibility genes account for approximately 40% of the excess

familial risk of EOC,[18] whereas rare moderate-risk variants and common low-risk variants contribute less than 5%.[15] Identification of additional susceptibility genes that confer RRs greater than 2 could decrease mortality as a result of ovarian cancer through surgical intervention (eg, risk-reducing salpingo-oophorectomy [RRSO]) in at-risk individuals. Recent advances in high-throughput next-generation sequencing technologies have enabled the rapid, targeted analysis of multiple candidate genes in large populations and have recently identified some novel susceptibility genes for ovarian cancer, including *RAD51C*,[19] *RAD51D*,[20] and *BRIP1*.[21] Existing data suggest that the population prevalence of germline mutations in these genes is low, but the published risk estimates (albeit on the basis of small sample sizes) suggest genetic testing of these genes may have clinical utility. *RAD51D* mutations were associated with a 6.3-fold increase in risk (95% CI, 2.9 to 14),[20] whereas *BRIP1* mutations were associated with an 8.1-fold increased risk of ovarian cancer (95% CI, 4.7 to 14).[21]

The aims of this study were to establish the prevalence and penetrance of deleterious mutations in the three interacting double-strand DNA break repair genes *RAD51B*, *RAD51C*, and *RAD51D*.

PATIENTS AND METHODS

Study Participants

The 3,447 confirmed invasive EOC cases and 2,812 unaffected controls were from four population-based ovarian cancer case-control studies (AOC [Australian Ovarian Cancer Study], MAL [Malignant Ovarian Cancer Study], SEA [Studies of Epidemiology and Risk Factors in Cancer Heredity], and UKO [United Kingdom Ovarian Cancer Population Study]), one clinic-based case-control study (MAYO [Mayo Clinic Ovarian Cancer Study]), one familial ovarian cancer series of cases and matched controls from Poland (POC [Poland ovarian cancer study]), and two familial ovarian cancer registries from the United Kingdom and United States (UKR [United Kingdom Familial Ovarian Cancer Registry] and GRR [Gilda Radner Familial Ovarian Cancer Registry]). These studies have been previously described (Table 1 and Appendix Table A1, online only). Forty-three duplicate samples and four *RAD51C* mutation–positive controls were included for quality control.

Also included were 2,000 unaffected participants enrolled onto the United Kingdom Familial Ovarian Cancer Screening Study (UK_FOCSS).[22] Eligible participants were women age ≥ 35, with an estimated lifetime risk of ovarian cancer of ≥ 10% on the basis of a family history of ovarian and/or breast cancer and/or the presence of known predisposing germline gene mutations (*BRCA1*, *BRCA2*, and *MMR* genes) in the family. Volunteers were recruited between June 2002 and September 2010 from 42 United Kingdom regional centers. All participants were tested for *BRCA1* and *BRCA2* mutations, and carriers were excluded from this study.

All studies had approval from the appropriate ethics committee, and all study participants provided written, informed consent.

Sequencing Library Preparation and Sequencing

We used the 48.48 Fluidigm Access Arrays (Fluidigm, San Francisco, CA) for target sequence enrichment, as described previously[8] and according to the manufacturer's protocol. The *RAD51* genes were in a panel of 11 genes sequenced in SEA and MAYO and in a panel of six genes in the remaining studies. The results for the other genes have been reported previously[8] or are unpublished. Fifty-six primer pairs were designed to cover the exons and splice sites of *RAD51B*, *RAD51C*, and *RAD51D* (Appendix Table A2, online only) with a combined sequencing target of 4 kb. The primer design achieved greater than 95% coverage of the target sequence. Sequencing libraries were quantified by using a KAPA library quantification kit (Kapa Biosystems, Boston, MA) with specific probes for the ends of the adapters according to the manufacturer's protocol. The sequence libraries were sequenced using single-end sequencing on the Illumina GAII (Illumina, San Diego, CA) or paired end sequencing on the Illumina HiScan (Illumina) or Illumina HiSeq 2000 (Illumina) according to the manufacturer's protocol. Each lane sequenced 384 barcoded samples.

Sequence Data Analysis

Sequenced reads were demultiplexed with standard Illumina software. We used the Burrows-Wheeler Aligner (http://bio-bwa.sourceforge.net/)[23] for sequencing read alignment against the human genome reference sequence (UCSC hg19; University of California Santa Cruz Genome Reference Consortium; http://genome.ucsc.edu/cgi-bin/hgGateway). The Genome Analysis Toolkit (GATK; https://www.broadinstitute.org/gatk/)[24] was used for base quality-score recalibration, local insertion/deletion (indel) realignment, and variant (substitution and indel) discovery. Variants were considered only if they satisfied the set of recommended GATK filters, as described in the GATK best practices guide. ANNOVAR (http://annovar.openbioinformatics.org/en/latest/)[25] was used to annotate the sequence variation detected. We used PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/bgi.shtml),[26] SIFT (http://sift.bii.a-star.edu.sg/),[27] and Provean (http://provean.jcvi.org/protein_batch_submit.php?species=human)[28] to predict the function of missense variants. We used MaxEntScan (http://genetics.bwh.harvard.edu/pph2/bgi.shtml)[29] to predict the pathogenic potential of possible splicing variants in sequences from 3 base pairs (bp) in the exon to 20 bp in the intron for the 3′ acceptor sites and 3 bp in the exon and 6 bp in the intron for the 5′ donor sites. Variants with a MaxEntScan score that decreased by more than 40% compared with the consensus sequence were assumed to affect splicing.

The alternate allele frequency (Altfreq) for each variant detected in each sample was defined as the fraction of alternative allele reads compared with the

Table 1. Study Patient Cases Sequenced for *RAD51B*, *RAD51C*, and *RAD51D* After Quality-Control Analysis

Study	Study Abbreviation	No. of Patient Cases	No. of Controls	Total No. of Participants
On the basis of patients not selected for family history				
Australian Ovarian Cancer Study[1]	AOC	413	428	841
Malignant Ovarian Cancer[1]	MAL	190	191	381
SEARCH[2]	SEA	1,259	1,382	2,641
United Kingdom Ovarian Cancer Population Study[1]*	UKO	361	531	892
Mayo Clinic Ovarian Cancer Study[2]	MAYO	912	146	1,058
Family based				
Poland family history, Poland ovarian cancer study[1]†	POC	89	94	183
United Kingdom Familial Ovarian Cancer Registry[3]†	UKR	48	—	48
Gilda Radner Familial Ovarian Cancer Registry[3]†	GRR	157	—	157
Total of all studies		3,429	2,772	6,201

Abbreviation: SEARCH, Studies of Epidemiology and Risk Factors in Cancer Heredity.
*Only study not screened for *BRCA1*/*BRCA2* mutations.
†All patient cases had a family history of ovarian cancer.

total number of reads at that position. We applied thresholds for variant calling, as defined previously[8]: With a minimum read depth of 15, alternate allele heterozygotes were called if the depth was ≥ 500 and the Altfreq was ≥ 10%; if the depth ranged from 250 to less than 500 and the Altfreq was ≥ 15%; if the depth ranged from 30 to less than 250 and the Altfreq was ≥ 20%; or if the depth ranged from 15 to less than 30 and the Altfreq was ≥ 30%. Samples with fewer than 80% of the target bases covered at a read depth of ≥ 15 (40 controls and 18 cases) were excluded. We defined deleterious variants as those predicted to result in protein truncation (frameshift indels, consensus splice site substitutions, and nonsense substitutions) or those missense mutations that have been previously reported as deleterious on the basis of in vitro analysis[19,30] or predicted by MaxEntScan to affect splicing.

Ninety percent of the target sequence bases had read depths ≥ 15. The coverage for the three genes is summarized in Appendix Table A2. Concordance for variants called in the 43 duplicate samples was 100%. Four *RAD51C* mutation–positive controls also were detected.

Mutation Validation

We visually inspected the sequence alignments for all of the called deleterious variants by using the Integrative Genomics Viewer (Broad Institute, Cambridge, MA; https://www.broadinstitute.org/igv/). We validated all deleterious variants by polymerase chain reaction amplification and Sanger sequencing.[31]

Statistical Methods

We tested for an association between deleterious mutations and ovarian cancer risk by using unconditional logistic regression adjusted for the country of origin (Australia, Denmark, Poland, the United Kingdom, and the United States). Odds ratios and associated 95% CIs also were calculated with data from the case-control studies that were not family based (AOC, MAL, MAYO, SEA, and UKO).

We estimated the cumulative risk of ovarian cancer with equation 1 by applying the estimated odds ratio (RR) to population incidence data for England from 2011[32]:

$$\text{Cumulative risk} = 1 - e^{(-\text{cumulative incidence})}$$

See the Data Supplement for a spreadsheet with calculations.

We identified multiple missense variants that have unknown functional effects on the protein. We excluded all missense variants that had a minor allele frequency (MAF) of greater than 1% from additional analyses, because large-scale genome-wide association studies have shown that the RR conferred by a common susceptibility allele are small (RR < 1.3) and thus not detectable by the smaller sample size of this targeted-sequencing study. The statistical power to detect single rare alleles by association, even if they confer larger risk (RR > 2), is still modest. Therefore, we used the rare admixture likelihood (RAML) burden test[33] to test for an association on a gene-by-gene basis. The RAML test combines the data for multiple variants and allows for alleles associated with either an increased or a decreased risk. We classified variants with an MAF ≤ 1% into three groups: deleterious variants as defined previously (these were excluded from the RAML analyses); variants predicted to have a damaging effect on protein function by at least two of three prediction tools (SIFT [score ≤ 0.05], PolyPhen-2 [classified as probably damaging/damaging], and Provean [score ≤ −2.5]); and variants with probable benign effects. Only patients who had a call rate greater than 80% for missense variants and variants that had a call rate greater than 80% and genotype frequencies consistent with the Hardy-Weinberg equilibrium ($P > 10^{-5}$) were included in these analyses.

RESULTS

Deleterious RAD51B, RAD51C, *and* RAD51D *Mutations in Ovarian Cancer Cases and Controls*

Sequence data for the coding regions and splice site boundaries of *RAD51B*, *RAD51C*, and *RAD51D* were available for 3,429 invasive EOC cases and 2,772 controls after quality control (Table 1). We identified 135 unique variants, of which eight (5.9%) were frameshift indels, 10 (7.4%) were nonsense substitutions, five (3.7%) were predicted splice site alterations, and 113 (78%) were missense substitutions. Of the 113 missense variants, one (*RAD51C* 428A>G) was deleterious,[30] 105 had an MAF less than 1%, and seven (5.1%) had an MAF greater than 1%.

Table 2. Mutation Carriers Identified in *RAD51B*, *RAD51C*, and *RAD51D* in Ovarian Cancer Patient Cases and Controls

Mutation Carrier Status	Controls		Patient Cases			
			All		Unselected for Family History	
	No.	%	No.	%	No.	%
Noncarrier	2,769	99.9	3,401	99.2	3,112	99.3
Mutation carrier						
Any mutation	3	0.11	28	0.82	23	
RAD51B	0	0	2	0.06	2	0.06
RAD51C	2	0.07	14	0.41	10	0.32
RAD51D	1	0.04	12*	0.35	11*	0.35

*One patient case carried two deleterious mutations.

We identified deleterious mutations in two cases for *RAD51B*, 14 cases and two controls for *RAD51C*, and 12 cases and one control for *RAD51D*. Of these, 23 deleterious mutation carriers were identified in 3,135 cases (0.73%) unselected for family history (Table 2 and Appendix Table A3, online only). One case had two deleterious mutations close to each other and in *cis* (G217X and Q219X) in *RAD51D*. The prevalence of deleterious mutations was significantly higher ($P < .001$) in cases (28 of 3,429; 0.82%) than in controls (three of 2,772; 0.11%). Eight deleterious mutations were detected in more than one individual. Three of these (*RAD51C* 732delT and A428G and *RAD51D* C898T) were identified in a case and a control. Of the 29 predicted deleterious variants in cases, 22 (76%) were frameshift indels or nonsense variants, six (21%) were splice site substitutions, and one (3.4%) was a missense variant previously reported as deleterious.[30]

We also evaluated the prevalence of *RAD51B*, *RAD51C*, and *RAD51D* variants in 2,000 individuals from UK_FOCSS. We identified 149 unique variants, of which three (2.0%) were frameshift indels, three (2.0%) were nonsense substitutions, two (1.3%) were predicted splice site alterations, and 141 (95%) were missense substitutions. Thirteen participants carried one of the eight different deleterious mutations in one of these genes (one in *RAD51B*, seven in *RAD51C*, and five in *RAD51D*). The overall prevalence (0.65%) was significantly greater than that of the general population controls ($P < .001$; Table 3).

Ovarian Cancer Risks Associated With RAD51B, RAD51C, *and* RAD51D *Mutations*

The odds ratio (adjusted for country of origin) associated with a deleterious mutation in any of the three genes was 8.1 (95% CI, 2.4 to 27; $P = .001$) for all ovarian cancer subtypes and 9.3 (95% CI, 2.7 to 32; $P < .001$) for the serous subtype. Gene-specific odds ratios (adjusted for country of origin) for all ovarian cancer subtypes were 5.2 for *RAD51C* (95% CI, 1.1 to 24; $P = .035$) and 12 for *RAD51D* (95% CI, 1.5 to 90; $P = .019$). Gene-specific odds ratios for the serous subtype were 7.4 for *RAD51C* (95% CI, 1.6 to 35; $P = .011$) and 12 for *RAD51D*

Table 3. Characteristics of the United Kingdom Familial Ovarian Cancer Screening Study Mutation Carriers

Gene	Mutation Information				Proband Characteristic		Family History			
							No. of Affected First-Degree Relatives		No. of Affected First- and Second-Degree Relatives	
	cDNA Change	Location	Protein Change	Predicted Effect	Ref. Age, Years	Breast Cancer (age in years)	Ovarian Cancer	Breast Cancer	Ovarian Cancer	Breast Cancer
RAD51B	854-2A>G	Intron 8	NA	Splicing	58	No	0	0	2	0
RAD51C	C97T	Exon 1	Q33X	Nonsense	31	No	1	1	2	1
RAD51C	158delC	Exon 2	S53fs	Frameshift deletion	69	No	2	0	2	0
RAD51C	C577T	Exon 4	R193X	Nonsense	46	No	0	0	0	1
RAD51C	C577T	Exon 4	R193X	Nonsense	46	No	1	0	2	0
RAD51C	C577T	Exon 4	R193X	Nonsense	41	No	1	0	2	0
RAD51C	731delT	Exon 5	I244fs	Frameshift deletion	51	No	1	0	3	0
RAD51C	731delT	Exon 5	I244fs	Frameshift deletion	64	Yes (57)	2	0	3	1
RAD51D	263 + 1G>A	Intron 3	NA	Splicing	53	No	1	0	4	0
RAD51D	C556T	Exon 6	R186X	Nonsense	62	No	1	0	3	2
RAD51D	C556T	Exon 6	R186X	Nonsense	62	Yes (52)	1	0	1	1
RAD51D	C556T	Exon 6	R186X	Nonsense	25	No	1	0	1	0
RAD51D	748delC	Exon 9	H250fs	Frameshift deletion	50	No	1	0	1	1

Abbreviation: Ref., reference.

(95% CI, 1.5 to 97; $P = .021$). The estimated average cumulative risks of ovarian cancer by age 50 were 1.3% (95% CI, 0.3% to 6.0%) for *RAD51C* and 3.0% (95% CI, 0.4% to 21%) for *RAD51D*. The equivalent risks by age 70 were 5.2% (95% CI, 1.1% to 22%) for *RAD51C* and 12% (95% CI, 1.5% to 60%) for *RAD51D*.

Clinicopathologic Characteristics Associated With RAD51B, RAD51C, *and* RAD51D *Mutations*

The clinical and histopathologic characteristics of all patient cases are listed in Appendix Table A1. Mutation carriers were more likely than noncarriers to have high-grade serous versus other histologic subtypes ($P = .046$; Table 4). Eighteen percent of mutation carriers were diagnosed at ages 40 to 49 years, and no mutation carrier was diagnosed with ovarian cancer before age 40 years (Table 4). Carriers of a mutation in any of the *RAD51* genes were more likely than noncarriers to have a family history of ovarian cancer, although this difference was not statistically significant (24% *v* 14%; $P = .16$ for all genes). The proportion of *RAD51C* mutation carriers with a family history was higher (36%; $P = .021$; Table 5). In UK_FOCSS participants, mutation carriers were also more likely than noncarriers to be associated with a family history of ovarian cancer (Table 3); 9 of 13 mutation carriers (69%) compared with 548 of 1,987 noncarriers (28%) had a family history comprising two or more ovarian cancer cases in first- or second-degree relatives ($P < .001$).

RAD51B, RAD51C, *and* RAD51D *Missense Variants and Ovarian Cancer Risk*

We used three bioinformatics tools (SIFT, PolyPhen-2, and Provean) to predict the effects on protein function of 112 missense variants. Thirty missense variants were classified as deleterious by all three tools, 12 missense variants by at least two of three tools, and 15 variants by one of three tools; 55 missense variants were predicted to be neutral by all three tools (Appendix Table A4). For the 38 missense variants with an MAF $\leq 1\%$ and predicted by at least two of three tools to have a functional effect, we compared the relative burden in cases and controls for each gene with the RAML test.[33] We found some evidence for an association of the rare missense variation in *RAD51C* with an increased risk of ovarian cancer for all ovarian cancer subtypes (RAML test $P = .029$), and the effect was stronger for the serous subtype (RAML test $P < .001$). We also found some evidence of an association of missense variants in *RAD51D* with an increased risk of serous ovarian cancer ($P = .012$). There was little evidence of an association of rare missense variants in *RAD51B* and *RAD51D* with all ovarian cancer subtypes or in *RAD51B* with serous ovarian cancer ($P > .05$).

Table 4. Mutation Status by Age at Disease Onset and Histologic Subtype in Patient Cases With Ovarian Cancer

Mutation Status	No. (%) of Patients by Age at Diagnosis, Years					Histology, No. (%)	
	< 40	40-49	50-59	≥ 60	Unknown	High-Grade Serous	Other
Noncarrier (n = 3,401)	165 (4.9)	514 (15)	1,073 (32)	1,642 (48)	7 (0.2)	1,786 (53)	1,615 (47)
Mutation carrier (n = 28)	0	5 (18)	11 (39)	12 (43)	0	20 (71)	8 (29)
RAD51B (n = 2)	0	0	0	2 (100)	0	1 (50)	1 (50)
RAD51C (n = 14)	0	4 (29)	5 (36)	5 (36)	0	10 (71)	4 (29)
RAD51D (n = 12)	0	1 (8.3)	6 (50)	5 (42)	0	9 (75)	3 (25)

Table 5. Mutation Status by First Degree of Family History of Breast and/or Ovarian Cancer in Patient Cases With Ovarian Cancer

Gene	No. (%) of Patient Cases by Family History			
	No FH (n = 2,307)	OvFH Only (n = 430)	BrFH Only (n = 467)	BrOvFH (n = 29)
Noncarrier	2,292 (71)	424 (13)	463 (14)	29 (0.90)
Mutation carrier				
Any	15 (60)	6 (24)	4 (16)	0
RAD51B	1 (100)	0	0	0
RAD51C	6 (43)	5 (36)	3 (21)	0
RAD51D	8 (80)	1 (10)	1 (10)	0

Abbreviations: BrFH, first degree of family history of breast cancer; BrOvFH, first degree of family history of both ovarian and breast cancer; no FH, no first degree of family history of breast or ovarian cancer; OvFH, first degree of family history of ovarian cancer.

DISCUSSION

To our knowledge, this study is the largest population-based ovarian cancer study to date to estimate the prevalence of mutations in the *RAD51B*, *RAD51C*, and *RAD51D* genes. Overall, 0.81% of EOC cases had a mutation in one of these three genes compared with 0.11% in controls. Our data suggest that both *RAD51C* and *RAD51D* are ovarian cancer susceptibility genes; however, *RAD51B* mutations are unlikely to contribute substantially to ovarian cancer risk.

Several other studies have reported on the prevalence of germline genetic variations in these genes (Appendix Table A5). However, for most of these, the ascertainment of cases was complex: several sequenced an affected proband (either breast or ovarian cancer) from a family with multiple cases of breast and/or ovarian cancer. Six studies sequenced *RAD51C* in ovarian cancer cases unselected for family history,[34-39] but only one of these carried out equivalent sequencing of controls.[34] Three studies sequenced *RAD51D* in unselected ovarian cancer cases,[36,40,41] but none of these sequenced the whole gene in controls. In these studies, the mutation frequency in cases ranged from 0.4% to 1.1% for *RAD51C* and 0.8% to 1.1% for *RAD51D*.

In this study, the mutation frequency in cases unselected for family history was 0.32% for *RAD51C* and 0.35% in *RAD51D*. These are likely to be underestimates of the true mutation frequencies. Our next-generation sequencing approach enabled rapid and high-throughput analysis of candidate genes in thousands of samples but did not provide complete coverage of all genes in all samples (mean coverage per sample, 90%). Also, we used polymerase chain reaction–based enrichment of candidate gene coding regions; any deleterious mutations occurring outside these regions (eg, large genomic deletions and rearrangements) would not have been detected. Finally, we did not include missense variants in our prevalence estimates, because we could not be certain of their pathogenicity in the absence of definitive functional assays. However, burden tests for *RAD51C* and *RAD51D* variants indicate that rare missense variants that are predicted to disrupt protein function are significantly more prevalent in cases than controls, which suggests that at least a proportion of these variants is deleterious.

High-grade serous ovarian cancer (HGSOC) is the most common ovarian cancer subtype, and mutations were more prevalent in patients with HGSOC (1.1%) than in other subtypes (0.49%). This finding, perhaps, is expected, because deficiency of double-strand DNA break repair by homologous recombination as a result of germline mutations in *BRCA1* or *BRCA2* also is associated with HGSOC.[8,42]

Although there are similarities in the functional mechanisms associated with the *RAD51* genes and *BRCA1*/*BRCA2*, the genetic epidemiology suggests there are also differences. For example, *BRCA1* and *BRCA2* mutations confer risks of both breast and ovarian cancer, but there is little evidence from other studies that *RAD51C* or *RAD51D* mutations confer increased risks of breast cancer. The location of truncating mutations in *BRCA1*/*BRCA2* is associated with variable risks of breast and ovarian cancer.[43,44] All except two of the predicted truncating mutations identified in *RAD51C* were located between amino acid 143 and 319 in a functional domain in the C terminus of the protein (residues 79 to 376).[45] This domain is important for forming the *RAD51B-RAD51C-RAD51D-XRCC2* and *RAD51C-XRCC3* complexes. Likewise, all of the deleterious mutations identified in *RAD51D* were clustered in the C-terminal region (residues 77 to 328), which affects binding to *RAD51C* and likely impairs double-strand DNA break repair[45] (Fig 1).

Our RR estimate for *RAD51D* is similar to that reported previously by Loveday et al[20] (6.3; 95% CI, 2.9 to 14) on the basis of the analysis of families with multiple cases of ovarian cancer. Our RR estimate for *RAD51C* is similar to those reported by Pelttari et al[35] (6.3; 95% CI, 1.2 to 35) for unselected ovarian cancer. The wide CIs of risk estimates for both genes suggest that caution needs to be applied if the genes are used clinically for genetic risk prediction. In addition, the fact that 18% of ovarian cancers in women carrying *RAD51C* and

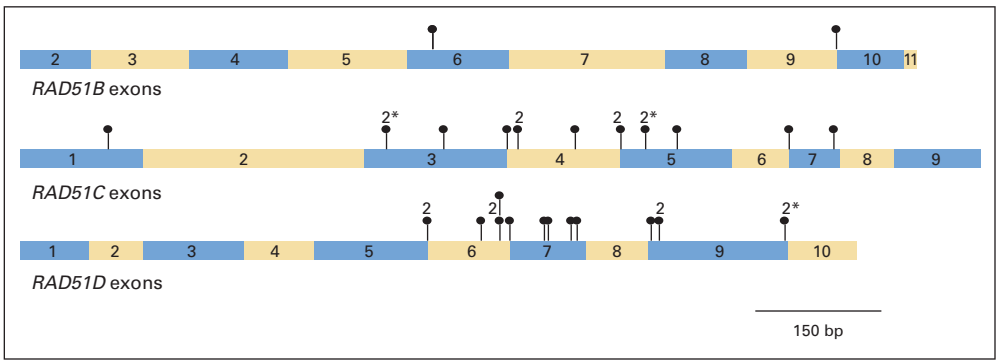

Fig 1. Distribution of predicted deleterious variants in *RAD51B*, *RAD51C*, and *RAD51D*. The location of each mutation is shown in the exon structure of the coding sequence. Mutations occurring in multiple individuals are indicated with the number of carriers above the small balloon. Coding regions of all the genes are on the same scale. (*) Deleterious mutation identified (one each in case and control groups). bp, base pair.

RAD51D mutations occurred at younger than 50 years (Table 4) suggests that, if risk estimates were confirmed, offering premenopausal women the option of RRSO should be considered. If clinical testing for *RAD51C* and *RAD51D* was approved, women could undergo panel testing for multiple susceptibility genes, and carriers, along with their relatives, could be offered RRSO.

In summary, we estimate that *RAD51B, RAD51C*, and *RAD51D* are responsible for approximately one in every 90 high-grade serous EOC occurrences and one in every 120 EOC occurrences. In addition to the benefit of mutation testing of *RAD51C* and *RAD51D* for disease prevention, mutation carriers also may be responsive to treatment with poly(ADP-ribose) polymerase inhibitors, which results in synthetic lethality of cells that have mutant homologous recombination or double-strand DNA break repair. This treatment might improve progression-free survival among these patients. Hence, such testing may be useful in patient decision making.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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GLOSSARY TERMS

allele: an alternative form of a gene (in diploids, one member of a pair) that is located at a specific position on a specific chromosome.

missense mutation: a change (mutation) in one nucleotide that results in the coding of a different amino acid.

penetrance: the likelihood that a given gene mutation will produce disease. This likelihood is calculated by examining the proportion of people with the particular genetic mutation that show symptoms of disease.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Contribution of Germline Mutations in the *RAD51B*, *RAD51C*, and *RAD51D* Genes to Ovarian Cancer in the Population

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Appendix

Table A1. Characteristics of the Patients With Ovarian Cancer

Characteristic	No. (%) of Patients by Study								No. (%) of Total Patients (N = 3,429)
	AOC (n = 413)	GRR (n = 157)	MAL (n = 190)	MAYO (n = 912)	POC (n = 89)	SEA (n = 1,259)	UKR (n = 48)	UKO (n = 361)	
Mean (range) age at diagnosis, years	60.1 (23-79)	49.4 (21-83)	61.7 (38-80)	62.5 (23-91)	51.1 (21-77)	56.0 (19-74)	53.0 (24-77)	61.2 (25-90)	58.7 (19-91)
Morphology									
High-grade serous	359 (87)	54 (34)	137 (72)	654 (72)	26 (29)	341 (27)	17 (35)	266 (74)	1,806 (53)
Low-grade serous	24 (5.8)	6 (3.8)	18 (9.5)	26 (2.9)	5 (5.6)	275 (22)	2 (4.2)	21 (5.8)	405 (12)
Serous	14 (3.4)	34 (22)	12 (6.3)	0	10 (11)	0	6 (13)	58 (16)	151 (4.4)
Endometrioid	3 (0.73)	19 (12)	13 (6.8)	110 (12)	13 (15)	214 (17)	5 (10)	6 (1.7)	383 (11)
Clear cell	3 (0.73)	12 (7.6)	6 (3.2)	55 (6.0)	1 (1.1)	144 (11)	2 (4.2)	2 (0.55)	225 (6.6)
Mucinous	2 (0.48)	8 (5.1)	2 (1.1)	25 (3.7)	9 (10)	116 (9.2)	3 (6.3)	1 (0.28)	166 (4.8)
Mixed	6 (1.5)	2 (1.3)	0	31 (3.4)	1 (1.1)	70 (5.6)	1 (2.1)	5 (1.4)	116 (3.4)
Other	1 (0.24)	21 (13)	0	11 (1.2)	24 (27)	79 (6.3)	11 (23)	2 (0.55)	152 (4.4)
Undifferentiated	1 (0.24)	1 (0.64)	2 (1.1)	0	0	20 (1.6)	1 (2.1)	0	25 (0.73)
Unknown	359 (87)	54 (34)	137 (72)	654 (72)	26 (29)	341 (27)	17 (35)	266 (74)	1,806 (53)
Stage*									
1	17 (4.1)	0	10 (5.3)	141 (15)	3 (3.4)	442 (35)	2 (4.2)	27 (7.5)	642 (19)
2	35 (8.5)	0	31 (16)	51 (5.6)	2 (2.3)	115 (9.1)	2 (4.2)	71 (20)	307 (9.0)
3	359 (87)	0	149 (78)	709 (78)	9 (10)	431 (34)	9 (19)	244 (67)	1,910 (56)
Unknown	2 (0.48)	157 (100)	0	11 (1.2)	75 (84)	271 (22)	35 (73)	19 (5.3)	570 (17)
Grade									
Low	28 (6.8)	18 (11)	42 (22)	128 (14)	12 (13)	411 (33)	7 (15)	24 (6.6)	670 (20)
High	370 (90)	74 (47)	135 (71)	754 (83)	41 (46)	670 (53)	28 (58)	275 (76)	2,347 (68)
Unknown	15 (3.6)	65 (41)	13 (6.8)	30 (3.3)	36 (40)	178 (14)	13 (27)	62 (17)	412 (15)

Abbreviations: AOC, Australian Ovarian Cancer Study; GRR, Gilda Radner Familial Ovarian Cancer Registry; MAL, Malignant Ovarian Cancer Study; MAYO, Mayo Clinic Ovarian Cancer Study; POC, Poland Ovarian Cancer Study; SEA, Studies of Epidemiology and Risk Factors in Cancer Heredity; UKO, United Kingdom Ovarian Cancer Population Study; UKR, United Kingdom Familial Ovarian Cancer Registry.
*Stages were defined as follows: 1, localized; 2, regional; and 3, distant.

Table A2. Sequencing Coverage by Gene

Gene	Accession No.	No. of Coding Exons	Total Coding Length (bp)*	No. of Amplicons Designed	% Coding Sequence* Covered by Design	Mean % Sequence Covered by Read Depth > 15
RAD51B	NM_133509	10	1,389	23	97	85
RAD51C	NM_058216	9	1,339	19	97	93
RAD51D	NM_002878	10	1,221	14	95	90

Abbreviation: bp, base pair.
*The sequence also contains 20 bp in the intron for the 3′ acceptor sites and 6 bp in the intron for the donor 5′ sites.

Table A3. Predicted Deleterious Mutations Found in *RAD51B, RAD51C*, and *RAD51D*

Study and Patient Group	Gene	cDNA Change	Location	Protein Change	Predicted Function	Ref. Age, Years	Ovarian Cancer FH1*	Breast Cancer FH1*	Grade Group	Histology Group
Control										
SEA	*RAD51C*	428A>G	Exon 3	Q143R	Missense	51	0	0		
SEA	*RAD51C*	732delT	Exon 5	I244fs	Frameshift deletion	56	0	0		
AOC	*RAD51D*	898C>T	Exon 9	R300X	Nonsense	49	0	1		
Patient case										
AOC	*RAD51B*	489T>G	Exon 6	Y163X	Nonsense	62			2	Serous HG
SEA	*RAD51B*	957G>C	Exon 9	Q319H	Splicing	61	0	0	2	Other
SEA	*RAD51C*	428A>G	Exon 3	Q143R	Missense	49	0	0	1	Serous LG
MAL	*RAD51C*	498delT	Exon 3	V166fs	Frameshift deletion	52	0	0	2	Serous HG
SEA	*RAD51C*	572-1G>T	Intron 3		Splicing	54	0	0	2	Serous HG
POC	*RAD51C*	577C>T	Exon 4	R193X	Nonsense	60				Other
POC	*RAD51C*	577C>T	Exon 4	R193X	Nonsense	41	1		2	Serous HG
AOC	*RAD51C*	653_654del	Exon 4	218_218del	Frameshift deletion	64	0	0	2	Serous HG
UKO	*RAD51C*	706-2A>G	Intron 4		Splicing	65	1	0	2	Serous HG
UKR	*RAD51C*	706-2A>G	Intron 4		Splicing	50	1		2	Serous HG
SEA	*RAD51C*	732delT	Exon 5	I244fs	Frameshift deletion	48	0	0	2	Serous HG
MAYO	*RAD51C*	774delT	Exon 5	R258fs	Frameshift deletion	55	0	1	2	Clear cell
POC	*RAD51C*	905-2delAG	Intron 6		Splicing	52	1			Endometrioid
AOC	*RAD51C*	955C>T	Exon 7	R319X	Nonsense	74	0	1	2	Serous HG
AOC	*RAD51C*	955C>T	Exon 7	R319X	Nonsense	40	0	1	2	Serous HG
SEA	*RAD51C*	97C>T	Exon 1	Q33X	Nonsense	61	0	0	2	Serous HG
UKR	*RAD51D*	478C>T	Exon 5	Q160X	Nonsense	56	1	0	2	Serous HG
MAL	*RAD51D*	564_567del	Exon 6	188_189del	Frameshift deletion	59			2	Serous HG
MAL	*RAD51D*	564_567del	Exon 6	188_189del	Frameshift deletion	76			2	Serous HG
SEA	*RAD51D*	564delT	Exon 6	T188fs	Frameshift deletion	59	0	0	2	Serous HG
SEA	*RAD51D*	576 + 1G>A	Intron 6		Splicing	66	0	0	2	Endometrioid
UKO	*RAD51D*	620C>A	Exon 7	S207X	Nonsense	59	0	0	2	Serous HG
SEA	*RAD51D*	623dupT	Exon 7	V208fs	Frameshift insertion	54	0	1	2	Serous HG
MAYO	*RAD51D*	655C>T/649G>T	Exon 7	G217X/Q219X	Nonsense	73	0	0	2	Serous HG
SEA	*RAD51D*	741_742insTG	Exon 9	T248_N249delinsX	Nonsense	56	0	0	2	Endometrioid
SEA	*RAD51D*	748delC	Exon 9	H250fs	Frameshift deletion	67	0	0	2	Serous HG
SEA	*RAD51D*	748delC	Exon 9	H250fs	Frameshift deletion	47	0	0	2	Serous HG
SEA	*RAD51D*	898C>T	Exon 9	R300X	Nonsense	62	0	0	2	Endometrioid

Abbreviations: AOC, Australian Ovarian Cancer Study; GRR, Gilda Radner Familial Ovarian Cancer Registry; MAL, Malignant Ovarian Cancer Study; MAYO, Mayo Clinic Ovarian Cancer Study; POC, Poland Ovarian Cancer Study; SEA, Studies of Epidemiology and Risk Factors in Cancer Heredity; Serous HG, high-grade serous; Serous LG, low-grade serous; UKO, United Kingdom Ovarian Cancer Population Study; UKR, United Kingdom Familial Ovarian Cancer Registry.
*First degree of family history.

Table A4. Catalog of Missense Mutations Found in *RAD51B*, *RAD51C*, and *RAD51D*

Gene and Variant Type	Chromosome	Position	cDNA	Exon	Protein	SIFT*	PolyPhen-2†	Provean‡	Score§	No. of Controls‖	No. of Patient Cases¶
Common variant (*MAF* ≥ 1%; n = 7)											
RAD51B	14	68352648	515T>G	Exon 6	L172W	1	1	0	2	64	69
RAD51B	14	68353893	728A>G	Exon 7	K243R	1	1	0	2	57	82
RAD51B	14	69061259	1094C>G	Exon 11	P365R	0	1	0	1	119	148
RAD51C	17	56772522	376G>A	Exon 2	A126T	0	0	0	0	31	37
RAD51C	17	56798128	859A>G	Exon 6	T287A	1	1	1	3	44	63
RAD51D	17	33433487	494G>A	Exon 6	R165Q	0	0	0	0	536	780
RAD51D	17	33430313	698A>G	Exon 8	E233G	0	1	1	2	90	124
Potentially deleterious rare variant (n = 38)#											
RAD51B	14	68331751	347A>G	Exon 5	Q116R	1	1	1	3	0	1
RAD51B	14	68331826	422T>A	Exon 5	I141N	1	1	1	3	1	0
RAD51B	14	68331829	425A>G	Exon 5	D142G	1	1	1	3	1	0
RAD51B	14	68352608	475C>T	Exon 6	R159C	1	1	1	3	0	2
RAD51B	14	68352609	476G>A	Exon 6	R159H	1	1	1	3	0	1
RAD51B	14	68352686	553T>G	Exon 6	C185G	0	1	1	2	1	1
RAD51B	14	68353814	649A>G	Exon 7	R217G	1	1	1	3	0	1
RAD51B	14	68878170	883G>A	Exon 9	A295T	1	1	1	3	0	1
RAD51B	14	68878171	884C>T	Exon 9	A295V	1	0	1	2	1	0
RAD51C	17	56770081	77A>T	Exon 1	K26M	1	1	1	3	0	1
RAD51C	17	56770084	80T>C	Exon 1	L27P	1	1	1	3	0	1
RAD51C	17	56772417	271C>T	Exon 2	L91F	1	1	1	3	0	1
RAD51C	17	56772481	335G>T	Exon 2	G112V	1	1	1	3	0	1
RAD51C	17	56772540	394A>C	Exon 2	T132P	1	1	1	3	0	1
RAD51C	17	56772543	397C>A	Exon 2	Q133K	1	1	1	3	0	1
RAD51C	17	56774068	419T>G	Exon 3	V140G	1	1	1	3	0	1
RAD51C	17	56774134	485G>A	Exon 3	G162E	1	1	1	3	1	0
RAD51C	17	56774146	497T>G	Exon 3	V166G	1	0	1	2	0	1
RAD51C	17	56780662	677T>C	Exon 4	L226P	1	1	1	3	0	1
RAD51C	17	56787260	746G>A	Exon 5	R249H	0	1	1	2	1	0
RAD51C	17	56787349	835G>C	Exon 5	A279P	1	1	1	3	0	1
RAD51C	17	56809885	1006A>C	Exon 8	T336P	1	0	1	2	0	1
RAD51D	17	33446607	26G>C	Exon 1	C9S	1	0	1	2	2	5
RAD51D	17	33445598	185C>T	Exon 3	S62L	1	0	1	2	1	0
RAD51D	17	33445581	202G>A	Exon 3	G68S	1	1	1	3	0	1
RAD51D	17	33434138	349T>A	Exon 5	C117S	1	1	1	3	0	1
RAD51D	17	33434081	406G>C	Exon 5	D136H	1	1	1	3	0	1
RAD51D	17	33433490	491T>C	Exon 6	L164P	1	1	1	3	0	1
RAD51D	17	33433488	493C>T	Exon 6	R165W	1	1	1	3	1	0
RAD51D	17	33433448	533T>G	Exon 6	M178R	1	0	1	2	1	1
RAD51D	17	33430511	629C>T	Exon 7	A210V	1	1	1	3	0	2
RAD51D	17	33430487	653G>A	Exon7	G218D	1	1	1	3	0	1
RAD51D	17	33430296	715C>T	Exon 8	R239W	1	1	1	3	0	1
RAD51D	17	33428338	785C>T	Exon 9	P262L	1	1	1	3	0	1
RAD51D	17	33428330	793G>A	Exon 9	G265R	1	1	1	3	2	0
RAD51D	17	33428309	814C>T	Exon 9	P272S	1	1	1	3	1	0
RAD51D	17	33428300	823C>T	Exon 9	R275W	1	1	1	3	0	1
RAD51D	17	33428015	944G>A	Exon 10	G315E	1	0	1	2	1	0
Probably benign rare variant (n = 67)											
RAD51B	14	68290285	25G>A	Exon 2	V9M	0	0	0	0	1	0
RAD51B	14	68290324	64C>T	Exon 2	H22Y	0	0	0	0	0	1
RAD51B	14	68292196	100T>C	Exon 3	S34P	0	1	0	1	1	0
RAD51B	14	68292283	187A>G	Exon 3	K63E	0	0	0	0	1	1
RAD51B	14	68301803	205G>A	Exon 4	G69R	0	0	0	0	2	0
RAD51B	14	68301820	222G>T	Exon 4	R74S	0	0	1	1	1	0
RAD51B	14	68301824	226G>A	Exon 4	A76T	0	0	0	0	0	1
RAD51B	14	68301830	232T>C	Exon 4	F78L	0	0	0	0	0	1
RAD51B	14	68301863	265G>A	Exon 4	A89T	0	0	0	0	0	1
RAD51B	14	68301872	274G>A	Exon 4	E92K	0	0	0	0	1	1
RAD51B	14	68301894	296C>T	Exon 4	A99V	1	0	0	1	0	1

(continued on following page)

Table A4. Catalog of Missense Mutations Found in *RAD51B*, *RAD51C*, and *RAD51D* (continued)

Gene and Variant Type	Chromosome	Position	cDNA	Exon	Protein	SIFT*	PolyPhen-2†	Provean‡	Score§	No. of Controls‖	No. of Patient Cases¶
RAD51B	14	68331763	359T>C	Exon 5	M120T	0	0	0	0	1	0
RAD51B	14	68331840	436G>A	Exon 5	A146T	0	1	0	1	1	1
RAD51B	14	68352659	526A>G	Exon 6	K176E	1	0	0	1	1	0
RAD51B	14	68352672	539A>G	Exon 6	Y180C	0	0	0	0	20	39
RAD51B	14	68353784	619G>T	Exon 7	V207L	0	0	0	0	17	22
RAD51B	14	68353913	748T>G	Exon 7	S250A	0	0	0	0	0	2
RAD51B	14	68878147	860C>A	Exon9	S287Y	1	0	0	1	0	1
RAD51B	14	68878180	893A>G	Exon 9	N298S	0	1	0	1	0	1
RAD51B	14	68878224	937C>G	Exon 9	L313V	0	0	0	0	0	1
RAD51B	14	68934949	1018G>C	Exon 10	E340Q	0	0	0	0	2	0
RAD51B	14	68934959	1028T>C	Exon 10	V343A	0	0	0	0	0	1
RAD51B	14	69061225	1060C>G	Exon 11	Q354E	0	0	0	0	1	0
RAD51B	14	69061226	1061A>C	Exon 11	Q354P	0	0	0	0	1	0
RAD51B	14	69061228	1063G>A	Exon 11	A355T	0	0	0	0	13	21
RAD51C	17	56770011	7G>A	Exon 1	G3R	0	0	0	0	0	1
RAD51C	17	56770018	14C>T	Exon 1	T5M	1	0	0	1	1	0
RAD51C	17	56770036	32A>G	Exon 1	Q11R	0	0	0	0	0	1
RAD51C	17	56770131	127C>T	Exon 1	P43S	0	0	1	1	0	1
RAD51C	17	56772345	199G>A	Exon 2	E67K	0	0	0	0	0	1
RAD51C	17	56772359	213T>A	Exon 2	N71K	0	0	0	0	1	0
RAD51C	17	56772390	244C>A	Exon 2	H82N	0	0	0	0	0	1
RAD51C	17	56772398	252G>T	Exon 2	K84N	0	0	0	0	0	1
RAD51C	17	56772504	358A>G	Exon 2	T120A	0	0	0	0	1	0
RAD51C	17	56774057	408G>A	Exon 3	M136I	0	0	0	0	1	0
RAD51C	17	56774080	431T>C	Exon 3	I144T	0	0	1	1	1	0
RAD51C	17	56774142	493A>T	Exon 3	M165L	0	0	0	0	1	2
RAD51C	17	56774155	506T>C	Exon 3	V169A	0	0	0	0	1	0
RAD51C	17	56774158	509T>G	Exon 3	V170G	0	0	1	1	1	1
RAD51C	17	56774170	521C>G	Exon 3	T174S	0	0	0	0	0	1
RAD51C	17	56774214	565G>A	Exon 3	G189R	0	0	0	0	0	1
RAD51C	17	56780592	607A>G	Exon 4	N203D	0	0	0	0	0	1
RAD51C	17	56780605	620A>G	Exon 4	H207R	0	0	0	0	0	1
RAD51C	17	56787298	784T>G	Exon 5	L262V	0	0	0	0	1	4
RAD51C	17	56787304	790G>A	Exon 5	G264S	0	0	1	1	19	23
RAD51C	17	56798141	872A>T	Exon 6	D291V	0	0	0	0	1	0
RAD51C	17	56801448	952G>A	Exon 7	D318N	0	0	0	0	0	1
RAD51C	17	56801452	956G>A	Exon 7	R319Q	0	0	0	0	1	1
RAD51C	17	56811513	1061C>T	Exon 9	A354V	0	0	0	0	0	1
RAD51C	17	56811542	1090A>G	Exon 9	S364G	0	0	0	0	1	0
RAD51D	17	33446566	67C>T	Exon 1	H23Y	0	0	0	0	1	1
RAD51D	17	33446143	131G>A	Exon 2	G44D	0	0	0	0	0	1
RAD51D	17	33446143	131G>C	Exon 2	G44A	0	0	0	0	0	1
RAD51D	17	33445575	208G>A	Exon 3	D70N	0	0	1	1	0	1
RAD51D	17	33434132	355T>C	Exon 5	C119R	0	0	0	0	2	2
RAD51D	17	33434093	394G>A	Exon 5	V132I	1	0	0	1	0	2
RAD51D	17	33433451	530A>G	Exon 6	Q177R	0	0	0	0	2	0
RAD51D	17	33433447	534G>C	Exon 6	M178I	0	0	0	0	1	0
RAD51D	17	33433446	535C>G	Exon 6	L179V	0	0	0	0	0	1
RAD51D	17	33433413	568G>A	Exon 6	A190T	0	0	0	0	0	2
RAD51D	17	33428370	753A>G	Exon 9	I251M	0	0	0	0	0	1
RAD51D	17	33428279	844G>A	Exon 9	E282K	0	0	0	0	0	1
RAD51D	17	33428261	862G>C	Exon 9	G288R	0	0	0	0	0	1
RAD51D	17	33428251	872G>A	Exon 9	R291H	0	0	0	0	1	0
RAD51D	17	33428245	878C>T	Exon 9	A293V	0	0	0	0	1	0
RAD51D	17	33428037	922A>G	Exon 10	M308V	0	0	0	0	0	1
RAD51D	17	33428022	937A>G	Exon 10	T313A	0	0	0	0	1	0

*SIFT: 0, tolerated; 1, not tolerated.
†PolyPhen-2: 0, benign/possibly damaging; 1, probably damaging.
‡Provean: 0, neutral; 1, deleterious.
§Score: number of algorithms (SIFT/PolyPhen-2/Provean) that predict deleterious effect of the missense variant.
‖No. of times the variant was identified in controls.
¶No. of times the variant was identified in controls.
#At least two of three prediction algorithms predict deleterious effect on protein function.

Table A5. Reported Targeted Sequencing on *RAD51* Genes

Study and Location by Gene	No. of Patients Analyzed						No. (%) of *RAD51* Mutations Identified					
	Total	BC	BC/OC	OC	uOC	Controls*	Total	BC	BC/OC	OC	uOC	Controls*
RAD51C												
Germany[19]	1,100	620	480	0	0	480 + 2,432†	6 (0.5)		6 (1.25)	0	0	0
Untied States (Zheng et al)‡	92	0	92	0	0	0	0	0	0	0	0	0
Canada (Akbari et al)‡	454	NS	NS	NS	0	0	0	NS	NS	NS	0	
Finland[35]	2,747	130 + 2,061†	139	8	409†	2,086†	8 (0.3)	0	2 (1.4)	2 (25)	4† (1.0)	2† (0.1)
Finland and Sweden[38]	1,704	1,105†	35	0	232 + 332†	871†	2 (0.1)	0	1 (2.8)	0	1 (0.4) + 0†	0†
United States (Clague et al)‡	286	133	34	119	0	0	0	0	0	0	0	0
Australia[37]	1,655	1,053	314	21	267	427	3 (0.2)	0	1 (0.3)	1 (4.8)	1 (0.4)	0
The Netherlands and Canada (De Leeneer et al)‡	351	0	239	112	0	0	0	0	0	0	0	0
Spain[30]	785	485	300	0	0	500	5 (0.6)	1 (0.2)	4 (1.3)	0	0	0
United States (Lu et al)‡	192	157	35	0	0	0	0	0	0	0	0	0
United Kingdom[34]	1,404	0	1,102	30	272	1,156	12 (0.9)	0	8 (0.7)	1 (3.3)	3 (1.1)	1 (0.09)
France (Coulet et al)‡	117	0	82	35	0	0	3 (2.6)	0	2 (2.4)	1 (2.9)	0	0
Germany (Schnurbein et al)‡	825	500	325	0	0	0	2 (0.3)	1 (0.2)	1 (0.3)	0	0	0
United States[36]	367	0	0	0	367	0	3 (0.82)	0	0	0	3 (0.82)	0
Spain (Blanco et al)‡	516	410	89	17	0	0	3 (0.6)	1 (0.24)	2 (2.2)	0	0	0
This study§	3,429	0	0	294	3,135	2,772	14 (0.41)	0	0	4 (1.4)	10 (0.32)	2 (0.07)
Total	16,024	6,654	3,266	636	5,014	10,724	61	3	27	9	22	5
Total fully sequenced				524	4,273	4,903				9 (0.017)	18 (0.004)	3 (0.0006)
RAD51D												
United Kingdom[20]	1,648	737	911	0	0	1,060		0	8 (0.88)	0	0	1 (0.09)
Canada and Belgium (Osher et al)‖	175	0	175	0	0	0	1 (0.57)	0	1 (0.57)	0	0	0
Finland (Pelttari et al)‖		2,200	95 + 297		541†	1,287†			2		3 (0.55)	0
United Kingdom[40]	1,305	741	303	16	245	466	2	0	0	0	2 (0.82)	0
Spain (Gutierrez-Enriquez et al)‖	713	171	491	51						4 (0.81)		
United States[36]	367	0	0	0	367	0	4	0	0	0	4 (1.1)	0
This study§	3,429	0	0	294	3,135	2,772				1 (0.34)	11 (0.35)	1 (0.036)
Total fully sequenced				361	3,747	4,298				5 (1.4)	17 (0.45)	2 (0.046)

Abbreviations: BC, breast cancer case proband from breast cancer familial study; BC/OC, breast and/or ovarian cancer proband from breast and/or ovarian cancer family; NS, not specified; OC, ovarian cancer proband from ovarian cancer family; uOC, ovarian cancer cases not selected based on family history.

*Unaffected controls.

†The subset was not fully sequenced but underwent genotyping for mutations detected previously.

‡*RAD51C* study references: Zheng et al: Breast Cancer Res Treat 124:857-861, 2010; Akbari et al: Breast Cancer Res 12:404, 2010; Clague et al: PLoS One 6:e25632, 2011; De Leeneer et al: Breast Cancer Res Treat 133:393-398, 2012; Lu et al: Fam Cancer 11:381-385, 2012; Coulet et al: Clin Genet 83:332-336, 2013; Schnurbein et al: Breast Cancer Res 15:R120, 2013; Blanco et al: Breast Cancer Res Treat 147:133-143, 2014.

§In the United States, United Kingdom, Australia, Denmark, and Poland.

‖*RAD51D* study references: Osher et al: Br J Cancer 106:1460-3, 2012; Pelttari et al: J Med Genet 49:429-432, 2012; Gutierrez-Enriquez et al: Int J Cancer 134:2088-2097, 2014.