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Frequency of mutations in a large series of clinically ascertained ovarian cancer cases tested on multi-gene panels compared to reference controls

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Abstract

Objectives—Given the lack of adequate screening modalities, knowledge of ovarian cancer risks for carriers of pathogenic alterations in predisposition genes is important for decisions about risk-reduction by salpingo-oophorectomy. We sought to determine which genes assayed on multi-gene panels are associated with ovarian cancer, the magnitude of the associations, and for which clinically meaningful associations could be ruled out.

Methods—7768 adult ovarian cancer cases of European ancestry referred to a single clinical testing laboratory underwent multi-gene panel testing for detection of pathogenic alterations in known or suspected ovarian cancer susceptibility genes. A targeted capture approach was employed to assay each of 19 genes for the presence of pathogenic or likely pathogenic alterations. Mutation frequencies in ovarian cancer cases were compared to mutation frequencies in individuals from the Exome Aggregation Consortium (ExAC). Analyses stratified by family and personal history of other cancers and age at diagnosis were also performed.

Results—Significant associations ($p < 0.001$) were identified between alterations in 11 genes and ovarian cancer, with eight of these displaying 5-fold increased risk (*BRCA1*, *BRCA2*, *BRIPI*, *MSH2*, *MSH6*, *RAD51C*, *RAD51D*). Relative risks of ovarian cancer greater than two-fold were also observed for *ATM*, but could reliably be ruled out for *RAD50* and *CHEK2*.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ygyno.2017.08.030>.

Conclusions—These results will inform clinical management of women found to carry pathogenic alterations in genes tested on multi-gene panels. The knowledge that some genes are not associated with OC can reduce concerns of women found to carry pathogenic alterations in those genes.

Keywords

Ovarian cancer; Multi-gene panel testing

1. Introduction

Ovarian cancer (OC) is the fifth leading cause of cancer death in U.S. women [1]. Because of the difficulties inherent in pre-symptomatic screening for OC, it is critically important to identify women at high risk of this disease who can be offered risk-reducing salpingo-oophorectomy (RRSO). Genetic screening is an important prevention tool for OC as RRSO in *BRCA1* and *BRCA2* mutation carriers is proven to reduce mortality [2]. Due to the large hereditary component of OC, multi-gene panel testing is commonly offered to women diagnosed with this form of cancer [3–6]. Equally important, relatives of women with OC who test negative for a pathogenic alteration can have some measure of assurance of lower personal risk.

Pathogenic mutations in *BRCA1* and *BRCA2* are found in 10–15% of unselected OC cases and account for up to 40% of heritable OC cases [7–11]. Several other genes have been associated with OC risk, such as *BRIP1*, *RAD51C*, and *RAD51D* [10,12–16]. However, the magnitude of the associations for these OC susceptibility genes is less well defined. In addition, it has been suggested that *PALB2* and *BARD1* confer increased risk of OC [12,13], but these findings need further evaluation. OC is also a well-established feature of Lynch syndrome that is associated with pathogenic alterations in the mismatch repair (MMR) pathway (*MLH1*, *MSH2*, *MSH6*, *PMS2*), but the gene-specific risks for OC with each of the MMR genes are not well defined [6,12,17,18]. Earlier studies of cancer predisposition genes involved in OC have been characterized by small sample sizes, a limited number of genes examined, or both. For example, Ramus et al. analyzed ~3200 cases and ~3400 controls but only examined four genes (*BRIP1*, *NBN*, *PALB2*, and *BARD1*) [13]. In contrast, Norquist et al. examined a larger set of genes in 1915 cases and reference controls [12].

In this study, we sought to determine the frequency of pathogenic alterations in a large series of OC cases referred for clinical testing and to provide estimates of OC risk associated with pathogenic alterations in genes commonly tested on multi-gene cancer panels.

2. Materials and methods

2.1. Study population

The data analyzed in this study were based on 10,203 adult (age at diagnosis ≥ 21) women with OC selected from 140,449 individuals referred to Ambry Genetics (Aliso Viejo, CA) for hereditary cancer multi-gene panel testing between March 15, 2012 and June 30, 2016. Test requisition forms were provided by the ordering clinician and, for the majority of

individuals, included details on patient demographics and clinical history including personal and family history of cancer, ages at diagnoses, along with tumor pathology for a subset of women.

Of the 10,203 OC cases, 7768 were Caucasian, including 7349 that self-identified as Caucasian and 419 that self-identified as Ashkenazi Jewish. The 7768 Caucasian individuals were tested on at least one of nine cancer panels offered at Ambry Genetics that include the majority of genes of potential relevance to OC (Supplemental Table 1).

The characteristics of the 7768 cases of OC included in the primary analyses are shown in Table 1. The median (range) age at diagnosis was 57.5 (21–90) years. Personal history of cancer other than ovarian was reported in 1992 (26%) cases, with breast cancer being the most frequent. The majority of women in the analyses (n = 6710 (86.4%)) also reported at least one first- or second-degree family member with a history of any cancer, with breast and colorectal cancers being the most common.

2.2. Multigene panel testing and sequence variant classification

Each of the multi-gene panels utilized in this cohort evaluates germ-line mutations using targeted custom capture and sequencing along with targeted chromosomal microarray analysis for copy number variant analysis as previously described [19]. In this study, germ-line genetic testing results were evaluated for 19 OC susceptibility genes. All variants identified were evaluated by a five-tier variant classification system by Ambry Genetics as previously described [20]. In primary analyses, we included variants classified as pathogenic or likely pathogenic (Supplemental Table 7). Ambry Genetics routinely submits variants and their classifications to the ClinVar database.

As previously shown in ovarian [12], breast [20] and prostate [21] cancer studies, the Exome Aggregation Consortium [22] (ExAC) dataset is an effective control dataset for the estimation of the gene-specific frequencies of pathogenic alterations. In this study OC cases were compared to non-Finnish European (NFE) controls from the ExAC dataset. Importantly, the dataset excluded germ-line variants found in exomes from The Cancer Genome Atlas (TCGA) to ensure to the extent possible that the ‘controls’ were cancer-free. Variants reported as PASS and non-PASS in ExAC were initially included in the dataset. Several of the non-PASS variants were observed in the OC cases, validated by Ambry Genetics, and classified as pathogenic or likely pathogenic. Therefore, restricting to PASS-only variants in the ExAC dataset was expected to inflate risk estimates for each gene. Variants reported as non-PASS were reviewed, and were excluded when observed at significantly different frequencies in other populations, genotyped in <20,000 controls, or called as multiallelic variants at the same position.

Variants in ExAC that were also observed by Ambry Genetics were classified based on the laboratory classification system. All other nonsense, frameshift, consensus dinucleotide splice site (± 1 or 2) were classified as pathogenic or likely pathogenic and were included in analyses. The remaining missense, splice site (± 3 + positions), synonymous, or intronic variants were classified as pathogenic/likely pathogenic when reported as pathogenic or

likely pathogenic by at least one clinical laboratory in ClinVar [23], with no conflicting reports (Benign/Likely Benign).

Following variant classification, some additional exclusions were applied. Variants with minor allele frequency (MAF) > 0.3% (except common founder mutations) in OC cases or ExAC controls were excluded from the study. Additionally, three individual variants associated with lower risks than the “average” pathogenic variant (*BRCA1**R1699Q; *CHEK2**I157T; *CHEK2**S468F) were excluded from both cases and controls. Protein truncating variants in the last exon or the last 55 bp of the penultimate exon were excluded from cases and controls due to influences of nonsense mediated mRNA decay, unless a known functional domain was disrupted. Potential mosaic variants in cases with an allele ratio > 70:30 were excluded from the case allele count. Large genomic rearrangements (LGRs) and variants in the *PMS2* pseudogene region (exons 9 and 11–15) were not included in risk analyses because these variants were not validated in ExAC; however these were included in the allele count for the case-case analyses.

2.3. Statistical methods

The frequencies of pathogenic/likely pathogenic (P/LP) alterations meeting criteria for each gene were compared to the frequencies in ExAC controls. Because individual data were not available in the ExAC data set we assumed that the observed frequency approximated a known population frequency and calculated a standardized risk ratio (analogous to a standardized incidence ratio using cancer registry data) by dividing the observed frequency in OC cases by the summed frequency of all qualifying P/LP alterations in the ExAC controls. p-Values were calculated using the Poisson distribution [24] and 95% confidence intervals were constructed using the Chi-Squared-based method [25]. A similar approach was used for analyses based on family history. We conducted case-case analyses of frequencies in earlier onset cases (<60 yrs) compared to frequencies in cases diagnosed at age 60 or older. The odds ratio (OR), p-values, and confidence intervals were calculated using STATA v14.0 [26]. Sensitivity analyses were performed limiting the analysis to pathogenic alterations only (i.e., excluding LP), including all ethnicities, and excluding cases where OC was not the first cancer diagnosed in cases (Supplemental Tables 2–4).

3. Results

Among the 7768 Caucasian OC cases and the 19 known/suspected OC susceptibility genes, 992 (12.8%) women harbored 1021 P/LP alterations. Twenty-eight (0.36%) women carried more than one P/LP variant. Of the 1021 P/LP alterations observed, 77 were large genomic rearrangements (LGRs), occurring most commonly in *BRCA1* (n = 28). For each of the genes evaluated, frequency of P/LP alterations is reported for Caucasian OC cases (Table 2) as well as African American, Asian, Hispanic, and Mixed Ethnicity (Supplemental Table 6).

Results comparing frequencies of P/LP alterations in OC cases and ExAC controls are shown in Table 2. As expected, genes that have well-established associations with OC such as *BRCA1*, *BRCA2*, *BRIP1*, *RAD51C*, and *RAD51D* exhibited significantly higher frequencies of P/LP alterations in OC cases than ExAC controls. Additionally, significant associations with OC were observed for mismatch repair (MMR) genes *MSH2* and *MSH6*.

Significantly ($p < 0.005$) elevated frequencies in OC cases compared to controls were also observed for *ATM*, *PALB2*, and *NBN*. Associations with OC were not observed for *MLH1* or *PMS2*, but these results should be interpreted with caution due to small numbers of mutations. Furthermore, associations with OC were not observed for *BARD1*, *CHEK2*, *MRE11A*, and *RAD50*.

Many of the genes tested in this study are associated with other cancers, most notably breast or colorectal cancer. To explore whether these associations were potentially confounding the OC results, we looked at subsets of OC cases that did not have a personal or family history of breast or colorectal cancer (Table 3). In general, this did not attenuate the significant SRRs; however, *PALB2* was no longer associated with OC when considering only cases without a personal or family history of breast cancer. Thus, the observed association between *PALB2* and OC in Table 2 was likely driven by the strong association of *PALB2* with breast cancer. To further explore this we compared the frequency of P/LP variants for *PALB2* in OC cases with and without a family history of BC. For example, ovarian cancer cases with *PALB2* mutations were 3.5× (OR = 3.5, 95% CI 1.1–14, $p = 0.017$) more likely to have a personal/family history of breast cancer than cases without such a history. In our view this further indicates that the increased frequency of *PALB2* mutations in our series is largely due to the well-established association of *PALB2* and breast cancer. That being said, we cannot conclusively rule out a modest independent effect of *PALB2* on ovarian cancer risk.

Because early onset disease is a common feature of many hereditary cancers, we examined the effect of age at diagnosis on mutation prevalence by comparing cases diagnosed before age 60 to those diagnosed at 60 and older (Table 4). As expected, *BRCA1*, *MSH6*, and *MSH2* P/LP alterations were observed at 3-fold higher frequencies in the earlier-diagnosed cases. In contrast, higher frequencies of P/LP alterations in *BRIP1* and *RAD51D* were observed in older OC cases. Although histopathology data were limited in this data set, we noted that 91 (91%) of the 100 ovarian cancers with known histopathology (excluding “mixed”, “other”, and “unknown”) and P/LP variants in *BRCA1*, *BRCA2*, *BRIP1*, *RAD51C*, *RAD51D*, were of serous type. In contrast, of the 17 epithelial ovarian cancers with such variants in the MMR genes (*MLH1*, *MSH2* and *MSH6*), seven (41%) were serous, 7 (41%) were endometrioid and 3 (18%) were clear cell tumors.

4. Discussion

To our knowledge, this is the largest study to date evaluating the frequency of P/LP alterations in clinical testing panel genes among OC cases. Our primary analyses include 7768 Caucasian OC cases compared to ~25,000 controls in the ExAC NFE population. The recent study by Norquist et al., was similar in design as it compared frequencies in a selected series of 1915 patients to ExAC reference controls. However, the Norquist et al. cases were ascertained from a variety of sources, with the majority coming from ongoing GOG clinical trials, and were unselected for age or family history. In contrast, our study was enriched for individuals with a personal/family history of cancer suggestive of hereditary risk, although the median age at diagnosis was only 3 years younger than the Norquist et al. study. In addition, a wider range of genes were assessed in the current study [12]. The larger size of

the cohort allowed for more precise estimation of relative risks, along with increased ability to detect genes with relatively modest associations with OC.

Our analyses confirmed previously identified OC susceptibility genes including *BRCA1*, *BRCA2*, *RAD51C*, *RAD51D*, and *BRIP1*. The SRRs reported for *BRCA1* (SRR = 11.78) and *BRCA2* (SRR = 7.96) were highly significant (Table 2), but lower than expected, likely due to inclusion of individuals who previously tested negative for *BRCA1* and *BRCA2* and returned for more comprehensive testing. The higher SRR for *BRCA1* is consistent with the established higher risk of OC in *BRCA1* carriers compared to *BRCA2* [6,27,28]. In addition, a higher frequency of *BRCA1* (but not *BRCA2*) P/LP alterations was observed in cases diagnosed before age 60 compared to older onset cases. We also confirmed the strong association of OC with P/LP alterations in *RAD51C* and *RAD51D* and provided a more precise estimate of risks associated with *RAD51C* because of the large sample size [12–15]. *BRIP1* was also associated with a high relative risk of OC despite a lower frequency of P/LP than previously reported [12,13]. The relative risks of OC associated with *RAD51C*, *RAD51D*, and *BRIP1* are of sufficient magnitude that RRSO might be offered to carriers of pathogenic alterations in these genes, consistent with NCCN guidelines.

Our large sample size allowed us to confirm associations for several suspected OC genes. In particular, we observed a highly statistically significant ($p = 1.84 \times 10^{-7}$) moderately increased frequency of P/LP alterations in OC cases compared to ExAC controls in *ATM*. Importantly, this was not attenuated when cases with personal or family history of breast cancer (Table 3) or pancreatic cancer (data not shown) were excluded, indicating an independent association between P/LP alterations in *ATM* and OC. In contrast, the increased frequency of P/LP alterations in *PALB2* in OC cases disappeared when breast cancer was removed (Table 3). We found some evidence for an elevated frequency of pathogenic alterations in *NBN* among cases as previously reported [12], although this was only marginally significant after Bonferroni correction for the number of genes tested. Although observed at low frequencies, *BARD1* has been suspected as an OC risk gene [12]. We did not observe an increased frequency of *BARD1* P/LPs among OC cases.

We identified 114 P/LP alterations in the four mismatch repair genes associated with Lynch syndrome including 48 in *MSH6*, 32 in *PMS2*, 28 in *MSH2*, and 6 in *MLH1* for a total frequency of 1.5%. Both *MSH2* and *MSH6* were associated with a significantly higher frequency in cases compared to ExAC controls (14-fold and 5-fold, respectively); these risks were attenuated, but still significant, when individuals with a personal or family history of colorectal cancer were removed from the analysis (Table 3). Only 22 and 33% of the OC cases in the primary analysis with P/LP alterations in MMR genes reported personal/family history that met the clinical definition of Lynch syndrome under the Amsterdam and Bethesda criteria, respectively (Supplemental Table 5), similar to findings from a recent study assessing MMR carriers with a broader phenotypic spectrum tested at the same laboratory [29]. Both *MSH2* and *MSH6* were highly associated with younger age at OC diagnosis (Table 4). Of the 715 cases that fit the Bethesda criteria for LS, 38 (5.3%) had P/LP alterations in one of the four MMR genes tested on the panel compared to 76 of 6691 (1.1%) of OC cases in our series that did not meet the Bethesda criteria.

We did not identify an increased risk of OC for either *MLH1* or *PMS2*. However, it should be noted that *PMS2* pseudogene regions (exons 9 and 11–15) were excluded from analysis because they were not validated in the ExAC dataset. Additionally, 44.9% of OC cases had a first- or second-degree relative with breast cancer, potentially influencing the results observed for *PMS2* and OC (Table 1). Therefore, *PMS2* in relation to OC risk should be further evaluated. *MLH1* was observed at a low frequency in this study (AF = 0.0004), which is consistent with previous studies [9, 10,12]. However, a previous study of Lynch syndrome families estimated the cumulative risk of OC by age 70 for *MLH1* mutation carriers at 20% [17], whereas the expected lifetime risk of a *BRCA1*-negative woman with a positive family history for OC is 2.6% [30]. Of the 6 *MLH1* mutation carriers in our cohort, 3 met Bethesda or Amsterdam II criteria for Lynch syndrome (Supplemental Table 5). Altogether, the current evidence suggests that *MLH1* mutation carriers need to be further investigated and carefully evaluated in relation to OC risk. Furthermore, our study suggests that gene specific and cancer site specific risks associated with the MMR genes needs to be evaluated more extensively. Current clinical management guidelines for Lynch syndrome suggest considering an RRSO for patients with a mutation in any Lynch syndrome gene [31]. This is of critical importance, as it is possible management guidelines related to RRSO may need to be altered on a gene by gene basis among the MMR genes.

The standardized risk ratios we calculated here can be considered estimates of the relative risk of OC averaged over all ages for a clinically tested population. However, it is important to note that determination of valid risk estimates will require retrospective or prospective analyses of risk in cohort or family studies. Nevertheless, the data presented here are important in determining which genes are associated with OC predisposition and the relative magnitudes of these associations in the context of this clinical cohort.

Two principal limitations associated with this study include the nonrandom ascertainment of the OC cases in the study and the use of ExAC controls for calculation of relative risks. Given the nature of this clinical series of index cases, which by definition qualified for genetic testing, the interpretation of the risk ratios reported in Table 2 are relevant specifically to a population referred for genetic testing based on a personal or family history of cancer. The use of ExAC non-TCGA controls was required in this study due to the lack of a series of ethnically matched female controls, and we have taken great care to ensure we have used the same criteria for pathogenicity of variants for the ExAC controls and the OC cases. Although the use of large reference datasets may not be ideal, the advantage of having such a large sample size allows precise estimates of the frequency of pathogenic alterations in a set of individuals without cancer. While there are a number of ongoing large case-control studies (combined ~70,000 cases and 70,000 controls) for breast cancer in which these genes will be sequenced, there are no such studies for ovarian cancer. Lastly, we note that even with the largest sample of ovarian cancer cases studied to date in a gene-panel testing context, pathogenic alterations in many of these genes were still sufficiently uncommon to prevent assessment of associations with risk (e.g., *MLH1*, *MRE11A*). Thus even larger studies or meta-analyses will be required to elucidate the relationship between sequence variation in these genes and OC.

This is the largest series of individuals with OC tested to date for a panel of cancer predisposition genes. The results will aid in clinical management of women and their relatives found to carry pathogenic alterations in associated genes as well as genes not associated with increased OC susceptibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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HIGHLIGHTS

- Ovarian cancer risks for mutations in hereditary cancer panel genes were assessed.
- Mutations by gene from 7768 ovarian cancer cases and reference controls were compared.
- BRCA1, BRCA2, BRIP1, MSH2, MSH6, RAD51C, and RAD51D were confirmed as high-risk genes.
- ATM was identified as a moderate risk ovarian cancer gene.
- The results will inform clinical management of women with mutations these genes.

Table 1

Characteristics of Caucasian individuals included in risk analyses.

			Caucasian Only analysis subset	
			n	%
Total patients			7768	
Ovarian cancer–age at diagnosis				
		<40	748	9.6
		40–49	1192	15.3
		50–59	2192	28.2
		60–69	2163	27.8
		70–79	1128	14.5
		80	273	3.5
		Unknown	72	0.9
Histopathology			1746	22.5
		Carcinosarcoma	24	1.4
		Germ cell	13	0.7
		Sex cord	54	3.1
		Other	20	1.1
		Epithelial	1637	93.8
		Serous	953	58.2
		Endometrioid	208	12.7
		Clear cell	152	9.3
		Mucinous	94	5.7
		Mixed	80	4.9
		Transitional cell	3	0.2
		Other	148	9.0
Personal history of other (non-ovarian) cancers ^a				
Breast	No		6666	86.1
	Yes		1073	13.9
Colorectal	No		7595	98.2
	Yes		142	1.8
Pancreatic	No		7703	99.6
	Yes		34	0.4
Endometrial	No		7232	93.5
	Yes		504	6.5
Family history of cancer (1st & 2nd degree only) ^a				
Ovarian	No		5911	84.9
	Yes		1049	15.1
Breast	No		3557	55.1
	Yes		2903	44.9
Colorectal	No		4878	70.1

		Caucasian Only analysis subset	
		n	%
Pancreatic	Yes	2082	29.9
	No	6274	90.1
Endometrial	Yes	686	9.9
	No	6391	91.8
	Yes	569	8.2

^aCategories are not mutually exclusive.

Table 2

Standardized risk ratio analysis of 7768 ovarian cancer cases compared to ExAC NFE-nonTCGA controls.

Gene	# of cases tested	Prevalence of mutations ^a	Case AC ^b	Case AF ^c	Control AF ^b	SRR	95% CI	p-Value
ATM	6315	0.87%	54	0.00428	0.00190	2.25	1.69–2.94	1.8×10^{-7}
BARD1	6294	0.14%	8	0.00064	0.00050	1.28	0.55–2.51	0.59
BRCA1	7489	3.97%	269	0.01796	0.00152	11.78	10.42–13.28	3.5×10^{-182}
BRCA2	7489	3.39%	250	0.01669	0.00210	7.96	7.00–9.01	2.3×10^{-99}
BRIP1	6294	0.99%	58	0.00461	0.00092	4.99	3.79–6.45	2.9×10^{-21}
CHEK2	6330	0.43%	58	0.00458	0.00465	0.98	0.75–1.27	0.87
MLH1	7411	0.08%	6	0.00040	0.00018	2.20	0.81–4.78	0.12
MRE11A	6294	0.11%	7	0.00056	0.00048	1.17	0.47–2.41	0.78
MSH2	7411	0.38%	23	0.00155	0.00011	13.91	8.82–20.87	2.5×10^{-17}
MSH6	7411	0.65%	47	0.00317	0.00063	5.04	3.70–6.70	1.7×10^{-17}
NBN	6294	0.38%	22	0.00175	0.00086	2.03	1.27–3.08	0.004
PALB2	6337	0.36%	22	0.00174	0.00056	3.08	1.93–4.67	1.2×10^{-5}
PMS2	7411	0.43%	14	0.00094	0.00064	1.48	0.81–2.48	0.2
RAD50	6294	0.25%	12	0.00095	0.00109	0.88	0.45–1.53	0.57
RAD51C	6294	0.79%	44	0.00350	0.00068	5.12	3.72–6.88	1.1×10^{-16}
RAD51D	5743	0.31%	11	0.00096	0.00015	6.34	3.16–11.34	4.4×10^{-6}

SRR: standardized risk ratio; AC: allele count; AN: allele number; AF: allele frequency.

^aIncludes LGRs, PMS2 pseudogene region, and variants excluded from ExAC dataset.

^bDoes not include LGRs, PMS2 pseudogene region, and variants excluded from ExAC dataset.

^cCalculated using Case AC and (# of Cases Tested * 2).

Table 3

Standardized risk ratio analysis excluding ovarian cancer cases with personal/family history of colorectal cancer or breast cancer.

Gene	ExAC-NFE controls ^a	Cases with personal/family history of colorectal cancer removed (n = 5590)						Cases with personal/family history of breast cancer removed (n = 3830)						
		Control AF	Case AC	Case AN	Case AF	SRR	95% CI	p-Value	Case AC	Case AN	Case AF	SRR	95% CI	p-Value
ATM	0.00190	36	9232	0.00390	2.06	1.44–2.85	0.0001	0.0001	28	6118	0.004577	2.41	1.60–3.49	6.3×10^{-5}
BRCA1	0.00152	190	10,824	0.01755	11.52	9.94–13.28	2.30×10^{-127}		90	7448	0.012084	7.93	6.38–9.75	1.0×10^{-47}
BRCA2	0.00210	189	10,824	0.01746	8.33	7.18–9.60	1.30×10^{-102}		103	7448	0.013829	6.60	5.38–8.00	8.9×10^{-60}
BRIP1	0.00092	45	9200	0.00489	5.30	3.87–7.09	1.30×10^{-17}		23	6104	0.003768	4.08	2.59–6.13	6.7×10^{-8}
MSH2	0.00011	9	10,576	0.00085	7.63	3.49–14.48	8.49×10^{-6}		6	7488	0.000801	7.18	2.64–15.63	0.0004
MSH6	0.00063	20	10,576	0.00189	3.00	1.83–4.64	4.50×10^{-5}		17	7488	0.002270	3.61	2.10–5.77	1.9×10^{-5}
NBN	0.00086	13	9200	0.00141	1.64	0.88–2.81	0.12		9	6104	0.001474	1.72	0.78–3.26	0.17
PALB2	0.00056	15	9270	0.00162	2.87	1.61–4.74	0.0007		4	6128	0.000653	1.16	0.32–2.97	0.91
RAD51C	0.00068	29	9200	0.00315	4.62	3.09–6.64	7.30×10^{-11}		20	6104	0.003277	4.80	2.93–7.42	3.9×10^{-8}
RAD51D	0.00015	9	8478	0.00106	7.02	3.21–13.34	1.63×10^{-5}		6	5658	0.001060	7.02	2.58–15.27	0.0005

SRR: Standardized Risk Ratio; AC: Allele Count; AN: Allele Number; AF: Allele Frequency.

^aExAC control AF used to calculate SRR, 95% CI, and p-value for both colorectal and breast cancer analyses.

Table 4

Ovarian cancer case-case analysis by age at diagnosis.

Gene	Cases < 60 at diagnosis (n = 4132)				Cases 60 at diagnosis (n = 3564)			
	OC cases tested	Cases with P/LP	% positive	OC cases tested	Cases with P/LP	% positive	OR	95% CI
ATM	3341	34	1.0	2916	19	0.65	1.57	0.87–2.92
BRCA1	3975	226	5.7	3451	70	2.0	2.91	2.21–3.88
BRCA2	3975	121	3.0	3451	129	3.7	0.81	0.62–1.05
BRIP1	3330	19	0.57	2906	42	1.4	0.39	0.21–0.69
MSH2	3928	25	0.64	3421	3	0.09	7.3	2.22–37.8
MSH6	3928	43	1.1	3421	5	0.15	7.56	3.00–24.5
NBN	3330	13	0.39	2906	11	0.38	1.05	0.41–2.71
PALB2	3350	10	0.3	2929	13	0.44	0.67	0.26–1.66
RAD51C	3330	29	0.87	2906	21	0.72	1.21	0.66–2.23
RAD51D	3020	5	0.17	2675	13	0.49	0.34	0.09–1.02