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Age-adjusted association of homologous recombination genes with ovarian cancer using clinical exomes as controls

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Abstract

Background: Genes in the homologous recombination pathway have shown varying results in the literature regarding ovarian cancer (OC) association. Recent case-control studies have used allele counts alone to quantify genetic associations with cancer.

Methods: A retrospective case-control study was performed on 6,182 women with OC referred for hereditary cancer multi-gene panel testing (cases) and 4,690 mothers from trios who were referred for whole-exome sequencing (controls). We present age-adjusted odds ratios (OR_{Adj}) to determine association of OC with pathogenic variants (PVs) in homologous recombination genes.

Results: Significant associations with OC were observed in *BRCA1, BRCA2, RAD51C* and *RAD51D*. Other homologous recombination genes, *BARD1, NBN,* and *PALB2,* were not significantly associated with OC. *ATM* and *CHEK2* were only significantly associated with OC by crude odds ratio (OR_{Crude}) or by OR_{Adj}, respectively. However, there was no significant difference between OR_{Crude} and OR_{Adj} for these two genes. The significant association of PVs in *BRIP1* by OR_{Crude} (2.05, CI = 1.11 to 3.94, $P = 0.03$) was not observed by OR_{Adj} (0.87, CI = 0.41 to 1.93, $P = 0.73$). Interestingly, the confidence intervals of the two effect sizes were significantly different ($P = 0.04$).

Conclusion: The lack of association of PVs in *BRIP1* with OC by OR_{Adj} is inconsistent with some previous literature and current management recommendations, highlighted by the significantly older age of OC onset for *BRIP1* PV carriers compared to non-carriers. By reporting OR_{Adj}, this study presents associations that reflect more informed genetic contributions to OC when compared to traditional count-based methods.

Keywords: Ovarian Cancer, BRIP1, Exome sequencing

Background

Next-generation sequencing (NGS) has enabled clinical laboratories to analyze simultaneously a growing number of genes. Clinical multi-gene hereditary ovarian cancer (OC) panels include genes that function in the same homologous recombination (HR) DNA repair pathway as *BRCA1* and *BRCA2*, such as *ATM, BARD1, BRIP1, CHEK2, NBN, PALB2, RAD51C,* and *RAD51D*. These genes have been linked to hereditary ovarian cancer, but the extent to which some of these genes contribute to

hereditary OC varies in the literature [1–7]. The identification of pathogenic or likely pathogenic variants (PVs) in these genes with ambiguous associations leads to difficult patient management decisions for clinicians and patients alike.

Identifying an appropriate control group for genetic association studies is vital for accurate estimation of cancer risks. Before high-throughput NGS, researchers relied on collaboration by pooling multi-center genotype data to achieve a large enough sample size to detect significant genetic associations [8, 9]. Recent association studies have drawn control data from the Exome Aggregation Consortium (ExAC), a public database which

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contains genotype information on over 60,000 individuals who have participated in genetic studies on conditions such as inflammatory bowel disease, heart disease, and schizophrenia [10–12]. The FLOSSIES database has also been used to perform genetic association studies specifically in women's cancer [13]. The advantage of such cohorts are the large, readily available number of genotyped individuals with diverse ancestries and varied clinical histories, but drawbacks include the lack of specific phenotypic information including age and cancer history of the participants.

Clinical laboratories are addressing this limitation by leveraging data from exome sequencing (ES) tests performed internally [14]. In our laboratory, ES can be performed on trios (child and both parents) to help identify variants contributing to rare Mendelian diseases. Healthy parents from these trios serve as a reliable control cohort for hereditary cancer studies considering the breadth of genotyping coverage and the adequate phenotypic information provided. We performed a case-control study with an internal control population and report age-adjusted ORs to clarify equivocal genetic associations in HR genes with OC.

Methods

Cohort assembly

Genotypic and phenotypic data were collected from women who underwent germline genetic testing at GeneDx (Gaithersburg, MD) between 2013 and 2018. The study was conducted in accordance with all guidelines set forth by the Western Institutional Review Board, Puyallup, WA (WIRB 20162523). Informed consent for genetic testing was obtained from all individuals undergoing testing, and WIRB waived authorization for use of de-identified aggregate data for both cases and controls. Individuals or institutions who opted out of this type of data use were excluded. Cases were women with OC referred for *BRCA1/2* alone or multi-gene hereditary cancer panel testing who did not also have a targeted test for a known familial variant. Controls were mothers from a subset of complete trios referred for ES due to a neurodevelopmental delay in the proband. These mothers all self-reported that they did not have a disorder with genetic etiology. Both case and control cohorts were limited to those over 18 years of age and those who self-reported as White/Caucasian. Women were excluded if they were missing age at diagnosis or age at testing or if they had more than one PV, including homozygous variants, in any of the cancer predisposition genes available on ordered panels (Additional file 1: Table S1). Of all individuals referred for multi-gene hereditary cancer panel testing, 9,688 women were diagnosed with OC, 6,182 of whom met inclusion criteria.

From the control cohort, there were 8,643 mothers of probands, of whom 4,690 satisfied the filtering criteria.
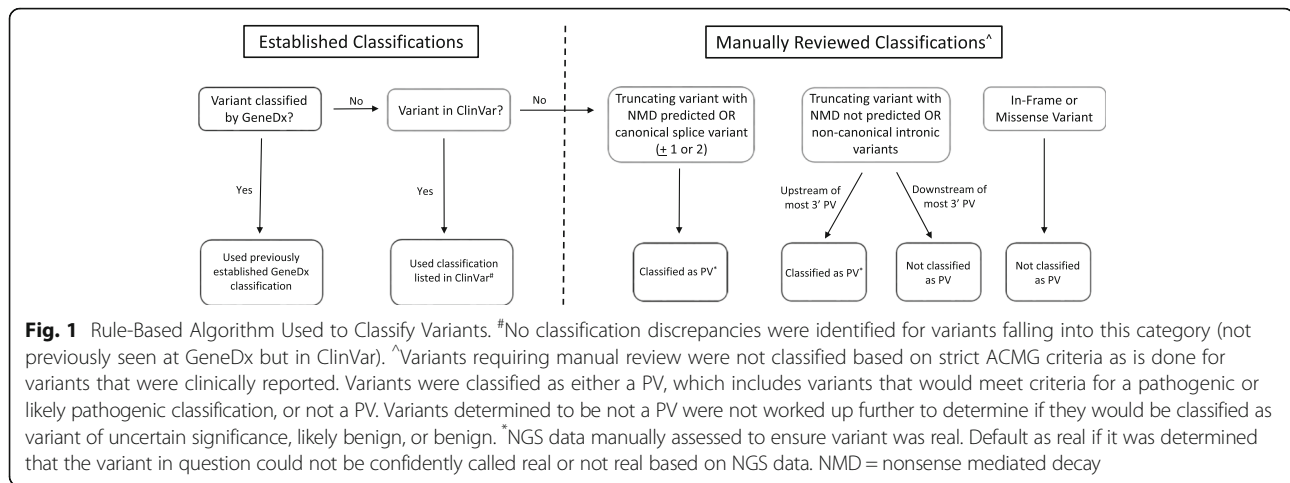
Sequencing and variant calling

Cases were sequenced and genotyped with targeted NGS panels and a clinical bioinformatics pipeline as previously described [15]. Controls were sequenced by clinical ES as previously described using either Agilent SureSelect Human All Exon v4 or Agilent Clinical Research Exome capture protocols (Agilent, Santa Clara, CA) [16]. Separate from clinical ES testing, control samples were jointly genotyped across the entire cohort following Genome Analysis Toolkit Best Practices using HaplotypeCaller (version 3.7.0) in GVCF mode followed by GenotypeGVCFs and variant quality score recalibration [17–19]. Single nucleotide variants with genotype quality less than 50 and insertions and deletions (indels) with genotype quality less than 99 were considered low confidence, and therefore not included in the analysis. Additionally, for all novel prospective PVs in controls, de-identified NGS alignment data were manually inspected to remove suspicious variants. Sequencing methods for cases and controls were evaluated for potential bias (Additional file 2: Additional Sequencing Methods and Table S2). Copy number variants were not evaluated as part of this study.

Variant classification

Analysis of genetic variants were limited to those located in the HR genes, *ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, NBN, PALB2, RAD51C,* and *RAD51D.* For cases, the pathogenic classification of each variant was reviewed according to internal protocol, which follows ACMG/AMP guidelines [20]. Exon-level deletions and duplications were not included in the PV count since detection of these copy-number variants from ES is more limited and variable than from panel testing. Pathogenic status of variants in controls were systematically classified using a rule-based algorithm (Fig. 1). Variants that were previously classified in cases were assigned the same classification. Novel variants in the controls were assigned ClinVar classifications or were manually classified when ClinVar classifications were not available or conflicting. ClinVar classifications were obtained from the variant call format file released September 5, 2017 [21]. The common founder pathogenic missense variants, *CHEK2*I157T and *CHEK2*S428F were excluded from both cases and controls. All PVs are listed in Additional file 3: Table S3.

Statistical analysis

A two-sample, independent t-test was performed to compare the mean age between cases and controls. Genetic associations were estimated by ORs using Firth bias-

Fig. 1 Rule-Based Algorithm Used to Classify Variants. #No classification discrepancies were identified for variants falling into this category (not previously seen at GeneDx but in ClinVar). ^Variants requiring manual review were not classified based on strict ACMG criteria as is done for variants that were clinically reported. Variants were classified as either a PV, which includes variants that would meet criteria for a pathogenic or likely pathogenic classification, or not a PV. Variants determined to be not a PV were not worked up further to determine if they would be classified as variant of uncertain significance, likely benign, or benign. *NGS data manually assessed to ensure variant was real. Default as real if it was determined that the variant in question could not be confidently called real or not real based on NGS data. NMD = nonsense mediated decay

corrected logistic regression [22]. Crude ORs (OR_{Crude}) were generated by simple logistic regression using status of PV in a gene as the independent variable. Multivariable logistic regression was performed with PV status and patient age (diagnosis in cases, at time of testing in controls) as independent variables to generate OR_{Adj}. Reported 95% confidence intervals (CIs) were calculated using penalized profile likelihood. A z-test was performed to compare OR_{Crude} and OR_{Adj} for each gene (Additional file 4). The aggregate PV prevalence per-gene was compared to reported prevalence from Lilyquist et al. using Fisher's exact test [23]. To correct for multiple independent tests, a Benjamini-Hochberg false discovery rate (FDR) correction was applied and a corrected *p*-value significance threshold of 0.05 was used when considering genetic association results and results from Fisher's exact test. The t-test and z-tests were two-sided and $P < 0.05$ were considered significant. A sensitivity analysis was performed by reporting OC associations in a subset of women who were diagnosed with serous OC subtype (Additional file 5: Table S4). Finally, a power analysis was performed using a test of proportions to determine the statistical power of the study. Conditions of the test used the total cohort sizes, assumed a significance level of 0.05, and a subjective, but realistic, assumption that rate of PVs in each cohort were 0.01, and 0.005, respectively. Statistical analyses were performed using the R programming language.

Results

Cohort summary
After filtering into final cohorts, the mean age (standard deviation) at OC diagnosis was significantly higher in cases than age at testing in controls (58.8 (13.4) versus 41.9 (9.4), $P < .05$, t-test). The median age at diagnosis of PV carriers in OC cases ranged from 53 years in *BRCA1* to 67 years in *BRIP1*, while the median age of testing of PV carriers in controls ranged from 32.5 years in *RAD51D* to 46 years in *BRIP1* (Table 1). There were 780

(12.6%) OC cases who also had a breast cancer diagnosis. Across all genes, the observed aggregate PV prevalence in either the case or control cohort were not significantly different, respectively, from those reported by Lilyquist et al. ($P = 0.05$, Fisher's exact test). There were 588 women of the 6,182 cases who harbored PVs (9.5%) while 98 of the 4,690 controls (2.1%) harbored PVs (Table 1).

Genetic associations
Well-established OC susceptibility genes showed significant associations when measured by both OR_{Crude} and OR_{Adj}: *BRCA1* (OR_{Crude} = 38.46, CI = 17.03 to 115.32, $P = 1.03 \times 10^{-47}$; OR_{Adj} = 47.80, CI = 20.76 to 145.03, $P = 1.87 \times 10^{-46}$), *BRCA2* (OR_{Crude} = 8.19, CI = 5.22 to 13.67, $P = 1.17 \times 10^{-28}$; OR_{Adj} = 5.10, CI = 3.12 to 8.83, $P = 2.89 \times 10^{-12}$), *RAD51C* (OR_{Crude} = 15.28, CI =3.97 to 137.01, $P = 6.09 x^{-06}$; OR_{Adj} = 12.09, CI = 2.78 to 114.43, $P = 7.85 \times 10^{-04}$), and *RAD51D* (OR_{Crude} = 7.99, CI = 2.56 to 39.77, $P = 1.97 \times 10^{-04}$; OR_{Adj} = 8.38, CI =2.17 to 47.23, $P = 2.51 \times 10^{-03}$) (Table 1).

Some genes with no significant association with OC measured by OR_{Crude} consistently showed no significant association with OC as measured by OR_{Adj}, including *BARD1* (OR_{Crude} = 1.59, CI = 0.31 to 9.56, $P = 0.57$; OR_{Adj} = 6.30, CI =0.55 to 74.25, $P = 0.19$), *NBN* (OR_{Crude} = 0.52, CI = 0.15 to 1.54, $P = 0.27$; OR_{Adj} = 0.45, CI = 0.10 to 1.82, $P = 0.33$), and *PALB2* (OR_{Crude} = 2.06, CI = 0.90 to 5.19, $P = 0.11$; OR_{Adj} = 1.78, CI = 0.61 to 5.59, P = 0.33).

ATM (OR_{Crude} = 2.01, CI = 1.15 to 3.64, $P = 0.03$; OR_{Adj} = 1.72, CI = 0.89 to 3.44, $P = 0.18$) and *BRIP1* (OR_{Crude} = 2.05, CI = 1.11 to 3.94, P = 0.03; OR_{Adj} = 0.87, CI =0.41 to 1.93, $P = 0.73$) were significantly associated with OC by OR_{Crude} but were not significantly associated with OC by OR_{Adj}. Conversely, *CHEK2* (OR_{Crude} = 1.62, CI = 1.00 to 2.69, $P = 0.07$; OR_{Adj} = 2.64, CI = 1.48

Table 1 Associations of ovarian cancer with pathogenic variants in homologous recombination genes among self-reported white women

Gene	Median Age of PV Carriers (Cases[a], Controls[b])	Crude Odds Ratio (CI)	p-value, Crude[c]	Adjusted Odds Ratio (CI)	p-value, Adjusted[c]	Case Carriers, No. (Non-Carriers)[d]	Control Carriers, No. (Non-Carriers)	p-value, z-test[e]
ATM	55.0, 47.0	2.01. (1.15 to 3.64)	0.03	1.72 (0.89 to 3.44)	0.18	35 (4724)	17 (4673)	0.35
BARD1	60.0, 34.5	1.59 (0.31 to 9.56)	0.57	6.30 (0.55 to 74.25)	0.19	3 (4122)	2 (4688)	0.80
BRCA1	53.0, 37.0	38.46 (17.03 to 115.32)	1.03×10^{-47}	47.80 (20.76 to 145.03)	1.87×10^{-46}	219 (5944)	4 (4686)	0.60
BRCA2	63.0, 40.0	8.19 (5.22 to 13.67)	1.17×10^{-28}	5.10 (3.12 to 8.83)	2.89×10^{-12}	193 (5969)	18 (4672)	0.14
BRIP1	67.0, 46.0	2.05 (1.11 to 3.94)	0.03	0.87 (0.41 to 1.93)	0.73	30 (4805)	14 (4676)	0.04
CHEK2	52.0, 42.0	1.62 (1.00 to 2.69)	0.07	2.64 (1.48 to 4.79)	2.36×10^{-3}	45 (4923)	24 (4287)	0.79
NBN	64.5, 37.0	0.52 (0.15 to 1.54)	0.27	0.45 (0.10 to 1.82)	0.33	4 (4232)	9 (4681)	0.46
PALB2	62.0, 39.0	2.06 (0.90 to 5.19)	0.11	1.78 (0.61 to 5.59)	0.33	16 (5004)	7 (4683)	0.50
RAD51C	61.0, 39.0	15.28 (3.97 to 137.01)	6.29×10^{-06}	12.09 (2.78 to 114.43)	7.85×10^{-04}	23 (4809)	1 (4689)	0.34
RAD51D	60.0, 32.5	7.99 (2.56 to 39.77)	1.97×10^{-04}	8.38 (2.17 to 47.23)	2.51×10^{-03}	20 (4809)	2 (4688)	0.44

[a]Age (in years) at time of ovarian cancer diagnosis
[b]Age (in years) at time of testing
[c]Corrected for False Discovery Rate
[d]All genes were not tested for every sample
[e]Comparing crude odds ratio to adjusted odds ratio
CI Confidence Interval, *No.* Number

to 4.79, $P = 2.36 \times 10^{-3}$) was not significantly associated with OC by OR_{Crude} but was significantly associated with OC by OR_{Adj}.

Power analysis and effect size comparison

The power analysis revealed that the study can detect a difference in proportions from 0.01 to 0.005 with 85.9% power. The per-gene comparison of effect sizes was not significantly different for the majority of the genes evaluated. The single exception was *BRIP1*, for which OR_{Adj} was significantly lower than OR_{Crude} (z-test, $P = 0.04$) (Table 1).

Discussion

Our approach is supported by the agreement of OR_{Crude} and OR_{Adj} with previously reported ORs for *BRCA1*, *BRCA2*, *RAD51C*, and *RAD51D*, genes with clearly established OC associations. The other HR pathway genes have been reported with conflicting evidence for association with OC [10, 14, 23–25]. Association of PVs in each these genes with OC are in agreement with at least one of these studies by either OR_{Crude} or OR_{Adj}. While this validates that our methodology is comparable, it also highlights the disparity of concordance among these studies.

To check for potential ascertainment bias, PV prevalence in cases and controls was compared to those previously reported by Lilyquist et al. [26] Given the similar ascertainment of cases and robustness of the estimates from the ExAC non-Finnish European control population, this data serves as a germane baseline for comparison. The prevalence of PV was not significantly different in any gene (Additional file 6: Table S5). Small

differences in PV counts due to differences in variant classification between studies can have a significant impact on effect size, especially for genes where PVs are rare. For example, Weber-Lassalle et al. reported 9 of 7,325 (0.12%) European PV carriers from the FLOSSIES database; however, our variant classification system would have reported 14 of 7,325 (0.19%), on the basis of including 5 European carriers of *BRIP1* c.139C > A (ClinVar: SCV000210833.12) (Fig. 1) [13].

Genes in which PVs are rare in one cohort (four or less PVs), *BARD1, BRCA1, NBN, RAD51C* and *RAD51D,* result in wide confidence intervals for the ORs. For example, *RAD51C* appears to confer greater risk than *BRCA2*, a gene whose association with OC has been well-established. However, with the exception of *CHEK2*, confidence intervals for ORs in all HR genes overlapped with those previously published, indicating the true effect size falls somewhere in the overlapping range [10]. Results from the power analysis can be interpreted that genes with moderate effect sizes are adequately powered to detect significant association but the result also supports the argument in favor of performing studies on genotyped cohorts large enough to sufficiently detect genetic associations in genes where PVs rare and the effect sizes are small.

No significant association with OC were consistently observed with PVs in *BARD1, NBN,* and *PALB2*. The reported low frequency of PVs in *BARD1*, similar to other studies, will require still larger sample sizes to detect a significant effect [10, 23, 24]. Association of *NBN* with OC is the low to moderate risk HR pathway gene with the most concordant results. It is widely regarded as not significantly associated with OC, with the exception of

Lilyquist, et al. who reported that its association was "marginally significant" [23]. Additionally, Lilyquist et al. initially reported a significant association of *PALB2* with OC, but the association was lost upon removal of women with a personal or family history of breast cancer [23]. We also report no significant association of *PALB2* with OC.

Multiple studies have reported *ATM* as a moderate risk OC gene [14, 23]. The observed significance by OR_{Crude} is concordant with these results. In controls, *ATM* PV carriers had the highest median age (47 years, time of testing) of any HR pathway genes. Given that PVs in *ATM* were only moderately associated with OC by OR_{Crude}, the median age of PV carriers was high enough compared to the median age of non-carriers to decrease the OR_{Adj} below the significance threshold. Notably, an insignificant z-test result indicates that the true effect size is likely to overlap the two reported CIs. The presence of undiagnosed cancer patients in the control cohort could falsely lower the calculated ORs, a similar effect was previously described in a study which used the ExAC controls with cancer samples included [10].

A well-known breast cancer risk gene, *CHEK2*, has been consistently reported to have no significant association with OC [10, 14, 23]. Similar to reported associations from *ATM*, the observed significance by OR_{Crude} is concordant with previously reported associations. In contrast, however, *CHEK2* PV carriers in cases had the lowest median age (52 years, time of OC diagnosis) of any of the HR pathway genes, which increased the OR_{Adj} enough to reach the level of significant association with OC. Again, similar to *ATM*, the z-test comparing the two effect sizes was insignificant, indicating that the true effect size is likely in the overlapping range of the CIs (Table 1). When limiting cases to women with serous OC pathology, association of OC with PVs in *CHEK2* disappears and the median age of OC diagnosis in PV carriers increases from 52 years to 63.5 years (Additional file 5: Table S4). None of the 12 *CHEK2* PV carriers with serous OC subtype were also diagnosed with breast cancer (not shown), suggesting that the significant OR_{Adj} result from the larger case cohort was a direct effect from the bias introduced by the younger *CHEK2* PV carriers who were also diagnosed with breast cancer.

Multiple publications have concluded that *BRIP1* is significantly associated with OC [10, 13, 23, 24]. While OR_{Crude} in *BRIP1* was significantly associated with OC, the OR_{Adj} showed no OC association. The significantly decreased effect size in *BRIP1* can be attributed to PV carriers displaying the oldest median age at time of OC diagnosis and the second oldest median age at time of testing among all of the genes (67y case PV carriers, 46y

control PV carriers; Table). Advanced age of PV carriers in *BRIP1* is not a novel observation [10, 13, 23, 24]. *BRIP1* was the only gene in which OR_{Adj} was significantly different than OR_{Crude}, which, given PV carriers are older compared to non-carriers, suggests that *BRIP1* PV carriers are more likely to be diagnosed with late-onset OC. Recent updates to National Comprehensive Cancer Network® (NCCN) guidelines for *BRIP1* PV carriers include consideration of risk-reducing salpingo-oophorectomy at 45–50 years of age [26]. The consistently reported older age at time of diagnosis and the observed lack of association by OR_{Adj} suggests caution before surgical intervention and the need for further studies of larger cohorts of older controls, as previously recommended [27].

A recent study reported no significant association of *BRIP1* with OC, but our results reveal a clinically relevant factor that offers insight which may have contributed to the observed lack of association. Age (mean [SD]) at time of OC diagnosis in cases (55.7y [14.1]) and age at time of testing in controls (39.7y [14.7]) were both younger than our cohorts, which could lead to undercounting *BRIP1* PV carriers. Finally, it has been suggested that *BRIP1*'s OC association may be restricted to high-grade serous epithelial ovarian cancer, but when cases were restricted to women diagnosed with high-grade serous OC histologic subtype, the OC association was consistent (Additional file 5: Table S4) [7].

By presenting OR_{Adj} in addition to OR_{Crude} this study allows for comparison of the two effect sizes. This comparison provides insight into the age difference between PV carriers and non-carriers and enables inference of early/late onset OC. Genes with PV carriers who are older than non-carriers demonstrate decreased OR_{Adj} compared to OR_{Crude} and conversely, genes with PV carriers who are younger than non-carriers have increased OR_{Adj} compared to OR_{Crude}. Comparing the two effect sizes for each gene using a z-test revealed that controlling for age did not significantly change the OR, except in *BRIP1*. A significant z-test result suggests that *BRIP1* PV carriers are more likely to be diagnosed with late-onset OC (Table 1).

As a referral laboratory, clinical information was limited to that provided by ordering providers with submitted samples. With ES, the referrals are not routinely submitted for cancer-related testing and, therefore, cancer history may not have been included in the clinical histories by the ordering physician. In addition, because the mean age of controls was lower than the mean age of OC diagnosis, it cannot be ruled out that some of these women will ultimately be diagnosed with OC. The OC referral cases could be biased toward a higher risk than the general ovarian cancer population. Variant detection sensitivity filters for controls were conservatively

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chosen which may lead to under-reporting of PVs and thus overestimation of ORs. Another source of possible under-reporting of PVs in controls is due to the incomplete gene coverage in controls, although the minor difference would have negligible effect (Additional file 2: Additional Sequencing Methods and Table S2). Copy number variants would have likely contributed a small number of PVs to cases and controls. But since this type of variant was not evaluated it acts as a source of bias toward under-reporting PVs. As not all PVs in controls were orthogonally confirmed as they were in cases, it is possible that a small number of control PVs were sequencing artifacts. Targeted *BRCA1* and *BRCA2* testing tends to have a lower positive yield compared to panel testing with other HR genes, as previously described [28]. The overall pathogenic variant rate in OC (9.5%) is lower than published rates from other studies [10, 23]. This can be attributed 873 women in the case cohort who underwent testing for *BRCA1* and/or *BRCA2* only.

Conclusions

We present age-adjusted genetic associations for PVs in HR genes with OC, leveraging an internal control cohort of women who self-report as White/Caucasian. Our study design and analysis provide more informed estimates of association compared to recently published OC associations by reporting both OR_{Crude} and OR_{Adj}. These results are most relevant for *BRIP1* PV carriers, as our findings for this gene are disparate from recent literature and conflict with current management guidelines.

Additional files

Additional file 1: Genes on GeneDx inherited cancer panels. (DOCX 13 kb)

Additional file 2: Additional sequencing methods and statistics. Details of GeneDx sequencing QC for comparison between cases and controls. (DOCX 13 kb)

Additional file 3: List of all pathogenic variants. The gene, cDNA, and protein change for each variant found in this study. (DOCX 57 kb)

Additional file 4: Calculation of the z-Statistic. The formula used to determine if crude odds ratios were significantly different than adjusted odds ratios. (DOCX 12 kb)

Additional file 5: Serous-subtype ovarian cancer sensitivity analysis. Results when limited to women with serous-subtype ovarian cancer. (DOCX 14 kb)

Additional file 6: Comparison of pathogenic variant prevalence with Lilyquist et al. Results from Fisher's Exact Test comparing prevalence of pathogenic variants in homologous recombination genes to Lilyquist et al. (DOCX 14 kb)

Abbreviations

ACMG: American college of medical genetics; AMP: Association for Molecular Pathology; CI: Confidence interval; ClinVar: Public database of reports of human variation; ES: Exome sequencing; ExAC: Exome aggregation consortium; FDR: False discovery rate; FLOSSIES: A sequencing database of women who are cancer free and over age 70; HR: Homologous recombination; NCCN: National comprehensive cancer network®; NGS: Next generation sequencing;

OC: Ovarian cancer; OR: Odds ratio; OR_{Adj}: Adjusted odds ratio; OR_{Crude} : Crude odds ratio; PV: Pathogenic variant

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