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...
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 GenDx (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*1157T 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-
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 × 10\(^{-47}\); OR_{Adj} = 47.80, CI = 20.76 to 145.03, P = 1.87 × 10\(^{-46}\)), BRCA2 (OR_{Crude} = 8.19, CI = 5.22 to 13.67, P = 1.17 × 10\(^{-28}\); OR_{Adj} = 5.10, CI = 3.12 to 8.83, P = 2.89 × 10\(^{-12}\)), RAD51C (OR_{Crude} = 15.28, CI = 3.97 to 137.01, P = 6.09 × 10\(^{-06}\); OR_{Adj} = 12.09, CI = 2.78 to 114.43, P = 7.85 × 10\(^{-04}\)), and RAD51D (OR_{Crude} = 7.99, CI = 2.56 to 39.77, P = 1.97 × 10\(^{-03}\); OR_{Adj} = 8.38, CI = 2.17 to 47.23, P = 2.51 × 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 74.23, 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
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 Liliquist 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

### 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, Controls) | Crude No. (Non-Carriers) | Control Carriers | p-value, Crude | Adjusted Odds Ratio (CI) | p-value, Adjusted |
|------|--------------------------------------------|--------------------------|-----------------|---------------|--------------------------|-----------------|
| ATM  | 55.0, 47.0                                 | 2.01 (1.11 to 3.64)      | 0.03            | 0.18          | 47 (724)                  | 0.35            |
| BARD1| 60.0, 34.5                                 | 1.59 (0.31 to 9.56)      | 0.57            | 0.19          | 3 (4122)                  | 0.80            |
| BRCA1| 53.0, 37.0                                 | 38.46 (17.03 to 115.32)  | 1.03 x 10^{-4}  | 0.57          | 47.80 (20.76 to 145.03)   | 1.87 x 10^{-6}  |
| BRCA2| 63.0, 40.0                                 | 8.19 (5.22 to 13.67)     | 1.17 x 10^{-2}  | 0.73          | 2.89 x 10^{-12}          | 0.14            |
| BRIP1| 67.0, 46.0                                 | 2.05 (1.11 to 3.94)      | 0.03            | 0.03          | 0.87 (0.41 to 1.93)       | 0.73            |
| CHEK2| 52.0, 42.0                                 | 1.62 (1.00 to 2.69)      | 0.07            | 0.33          | 2.64 (1.48 to 4.79)       | 0.29            |
| NBN  | 64.5, 37.0                                 | 0.52 (0.15 to 1.54)      | 0.27            | 0.27          | 0.45 (0.10 to 1.82)       | 0.33            |
| PALB2| 62.0, 39.0                                 | 2.06 (0.90 to 5.19)      | 0.11            | 0.33          | 1.78 (0.61 to 5.59)       | 0.33            |
| RAD51C| 61.0, 39.0                                | 15.28 (3.97 to 137.01)   | 6.29 x 10^{-6}  | 0.03          | 12.09 (2.78 to 114.43)    | 0.03            |
| RAD51D| 60.0, 32.5                                | 7.99 (2.56 to 39.77)     | 1.97 x 10^{-3}  | 0.03          | 8.38 (2.17 to 47.23)      | 0.03            |

aAge (in years) at time of ovarian cancer diagnosis
bAge (in years) at time of testing
cCorrected for False Discovery Rate
dAll genes were not tested for every sample
eComparing crude odds ratio to adjusted odds ratio
fConfidence Interval, No. Number
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 under-counting 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
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<sub>Crude</sub> and OR<sub>Adj</sub>. 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 Lillyquist et al. Results from Fisher’s Exact Test comparing prevalence of pathogenic variants in homologous recombination genes to Lillyquist 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; 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<sub>Adj</sub>: Adjusted odds ratio; OR<sub>Crude</sub>: Crude odds ratio; PV: Pathogenic variant

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**Author’s contributions**
All authors participated in some part of the study. KA, KR, RT, and LS designed the study. KA drafted the manuscript with contributions from MR, LS, RT, MM, KR, and ZZ. KA, RT, LS, MR, and MM performed data processing of the case cohort. ZZ performed data processing of the control cohort. KA, MR, LS, MM, LY, and NC reviewed relevant literature. KA and RT performed statistical analyses. MR and ER manually reviewed variants. KR, KH, and RK provided supervision and critical review of the study. All authors read and approved the final manuscript.

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**Availability of data and materials**
The datasets generated and/or analysed during the current study are not publicly available due to patient privacy restrictions but are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**
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.

**Consent for publication**
Not Applicable

**Competing interests**
The following individuals are employed by GeneDx, Inc., a wholly owned subsidiary of OPKO Health, Inc.: Arvai, Roberts, Toerne, Susswein, Marshall, Zhang, Carter, Yackowski, Rinella, Hruska, and Retterer. In addition, Dr. Hruska and Mr. Retterer have stock in OPKO Health, Inc. to disclose. Rachel Klein has stock options and employment with MyGeneTeam/OPKO Health, and indirect contractual relationships with GeneDx/BioReference Laboratories.

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