Author’s response to reviews

Title: Age-Adjusted Association of Homologous Recombination Genes with Ovarian Cancer Using Clinical Exomes as Controls

Authors:

Kevin Arvai (karvai@genedx.com)
Maegan Roberts (mroberts@genedx.com)
Rebecca Torene (rtorene@genedx.com)
Lisa Susswein (lsusswein@genedx.com)
Megan Marshall (mmarshall@genedx.com)
Zhancheng Zhang (zzhang@genedx.com)
Natalie Carter (ncarter@genedx.com)
Lauren Yackowski (lyackowski@BioReference.com)
Erica Rinella (erinella@BioReference.com)
Rachel Klein (rklein@genpathdiagnostics.com)
Kathleen Hruska (KHruska@genedx.com)
Kyle Retterer (KRetterer@genedx.com)

Version: 1 Date: 21 Jun 2019

Author’s response to reviews:

Response to the Reviewers Letter for Arvai et al.

Reviewer reports:

Having assessed the manuscript and taking into consideration the responses from the reviewers, the authors much address questions about the statistical methods used in the study and they must address the issues raised about the differences in the average age of the control group.
Response:

We thank the reviewers and editors for their thorough reading of the manuscript and have made revisions based on the valuable critiques. We performed an a priori power analysis and added to the discussion of confidence intervals overlapping with prior studies. Additional commentary were added regarding the inclusion criteria for the women in the control cohort.

We have also addressed minor critiques, highlighted by an accurate description of association of pathogenic variants in ATM with ovarian cancer, a revised Supplementary Table 3 to follow HGVS guidelines, and, finally, text in the Discussion with rationale for excluding copy number variants and clarification of the overall pathogenic variant rate in ovarian cancer.

Reviewer #1: In the paper by Arvai et al., the authors perform a retrospective case-control study on 6,182 women with ovarian cancer examined by multi-gene panel testing and 4,690 mothers from trio WES analysis, and present age-adjusted odds ratios to determine the association of ovarian cancer with pathogenic variants in ten homologous recombination genes. The authors confirm the association for pathogenic variants in BRCA1, BRCA2, RAD51C and RAD51D with ovarian cancer, while pathogenic variants in BARD1, NBN and PALB2 were not significantly associated with ovarian cancer. Moreover, ATM, CHEK2 and BRIP1 pathogenic variants were only significantly associated with ovarian cancer by crude odds ratio or by adjusted odds ratio. Arvai et al., conclude that their study design and analysis provide more informed estimates of association compared to recently published ovarian cancer associations by reporting both crude and adjusted odds ratio. The results are most relevant for BRIP1 pathogenic variant carriers as their findings regarding this gene is inconsistent with previous studies and current management recommendations.
Taken together, the manuscript is well-written, and the data is clearly presented.

Minor points

1) The authors should state in the method or in the beginning of the results section which HR genes were included in the analysis (genes in Table 1).
   • Thank you for the suggestion, we have added a sentence at the beginning of the Genetic Associations sub-section of the Results.

2) In line 108 (page 5), please include Supplementary Table 3 for uniformity.
   • Thank you for the suggestion, we have updated the text to include a more explicit reference to Supplementary Table 3.

3) In the last section on page 7, the authors mention that one gene, BRIP1, was significantly associated with OC by ORcrude. However, ATM was also significantly associated with OC by ORcrude. Should be rewritten.
   • Thank you for pointing out this mistake, we clarified the last paragraph of the Genetic Associations subsection in the Results where there was confusing language describing which genes were significantly associated by crude odds ratio and not significantly associated by adjusted odds ratio.

4) The reference list should checked. It seems that several references lack information regarding volume and page numbers.
   • Thank you for bringing this to our attention. The References section has been updated and now follows Vancouver citation style, per journal guidelines.

5) In Supplementary Table 3, information regarding nucleotide change is missing for several variants. Moreover, please use either Ter or * (not X) for nonsense variants at the protein level (follow the HGVS guidelines). Moreover use * for frameshift variants (not X). Finally, regarding ATM c.7636_7644del, use HGVS guidelines for protein nomenclature.
   • Thank you for bringing this to our attention, we have revised Supplementary Table 3 to follow HGVS guidelines.
6) I would suggest that the authors update variant classification in ClinVar for the pathogenic variants listed in Supplementary Table 3, e.g. ATM c.3848T>C, p.Leu1283Pro (classified by GeneDX in 2015 as a variant of unknown significance).

- Thank you for noticing the discrepancy, GeneDx submitted a request to ClinVar for this variant’s classification to be updated from Variant of Unknown Significance to Likely Pathogenic.

Reviewer #2: The work submitted by Arvai et al performs a case-control study in an effort to provide estimates on ovarian cancer risk associated with pathogenic variants in a number of genes sequenced.

I think that these type of data are extremely important since valuable results can emerge in respects to patient clinical management, as well as therapeutic choices. Most works published so far use data from public databases, such as ExAC, to perform statistical associations. Therefore, the backbone of this study is relatively novel and can be valuable.

Although I don't have a major in Statistics, I am a little concerned about the "control" group. The total number of controls used was 4690, while the number of patients used was significantly larger (6182).

- Thank you for raising this point, when cases outnumber controls it can affect the statistical power of the study. For this reason, we performed an a priori power analysis to determine the statistical power. It is difficult to estimate power for the study in its entirety, given that each gene was tested for association independently with different cohort sizes and unknown effect sizes. Knowing this, we calculated power using a test of proportions solving for power with subjective pathogenic variant rates of 0.01 and 0.005 between two cohorts.

The results have been included in the Power Analysis and Effect Size Comparison subsection of the Results section. The published power of 85.9% is not appreciably lower than the power calculation if we changed the assumption that the number of controls had been equal to the number of cases (90.4%, not included in the manuscript).
But, the major issue is the relatively young age of the controls. Considering that even in BRCA1/2 carriers, the mean age at ovarian cancer diagnosis is ~51 years, the authors state the mean age in their control group was 41.9 years. This means that we cannot actually rule out that some of these women will ultimately be diagnosed with ovarian cancer. FLOSSIES, on the contrary has such data, since all the women included in the study were all over 70 years. Since the number of ovarian cases genotyped herein is relatively large, I feel that the best way out is to compare these data with data from FLOSSIES and/or ExAC.

- We thank the reviewer for these critiques. We should have included in the manuscript that the women from the control cohort were selected because they self-reported as not having a disorder with a genetic etiology. While this does not rule out the chance for these women to develop ovarian cancer, to the best of our knowledge, we believe that the cohort is currently cancer-free. We have added this detail to the Methods section.

The novelty of the study was to show that using internal controls can not only lead to reproducible genetic associations, but also reveal interesting new findings, previously unattainable because of limitations from public datasets. We were encouraged by the reproduced genetic associations when comparing our results to Lilyquist, et al. and Norquist, et al. both of which used the exome aggregation consortium database as a control cohort. With a discrepant association, such as with BRIP1, we felt it was appropriate to provide commentary that advanced age seemed to contribute to the outcome of ovarian cancer in the BRIP1 test significantly more than in tests of other HR genes.

Minor Comments

- There are some inconsistencies throughout the manuscript, but foremost the Results presented in Table 1 are rather misleading; it seems that RAD51C PVs confer higher risk than BRCA2 PVs, while the authors comment that their results are comparable to previous studies.

- Thank you for raising these concerns, we have corrected the inconsistencies in the Genetic Associations subsection in the Results section, as also pointed out by Reviewer 1. With respect to the appearance of RAD51C conferring higher risk than BRCA2, it is notable that pathogenic variants in RAD51C were extremely rare in controls which resulted in wide confidence intervals for the effect sizes. We have included more robust commentary surrounding confidence intervals in the Discussion, which should address these concerns.
-CNVs are excluded from analysis and this should be highlighted and mentioned in the Discussion.

-Thank you for pointing out this important omission, not investigating copy number variants was a limitation of the study. The last sentence of the Sequencing and Variant Calling subsection of the Methods section describes the omission of this variant type from the study. We have updated to Discussion to acknowledge this variant type as a source of pathogenic variation in HR genes. Many of the studies we cite published their pathogenic variant frequency without copy number variants as well, and this is a potential limitation of all of these studies.

-The mutation rate, i.e. 9.2%, is quite lower that those already reported and there should be a comment on that.

-Thank you for highlighting this discrepancy between our pathogenic rates and the published pathogenic rates from cited studies. Women from the case cohort underwent targeted genetic testing. There were 873 women who were only tested for BRCA1 and/or BRCA2. Targeted tests which interrogate only these two genes have a lower positive yield when compared with panel testing where more HR genes are interrogated. The last paragraph of the Discussion includes a brief description of these points and cites a recent manuscript which further characterizes the GeneDx hereditary cancer testing population.

-Exome seq Vs panel seq has differences in the sensitivity of variant detection; again this should be mentioned in the Discussion.

-Thank you for highlighting the difference in sequencing technologies between cohorts. A detailed description found in Additional Sequencing Methods from Additional file 2 was omitted from the main manuscript. We have added this reference for clarity.

-The two ORs is rather confusing and difficult to follow.

-Thank you for this critique. We acknowledge that readability suffers as a result of explicitly referring to crude and adjusted odds ratios throughout the text. However, our conclusion was dependent upon having both odds ratios so we could elucidate the effect of age of diagnosis. We recommend that readers refer to Table 1 to compare the two odds ratios.