Author's response to reviews

Title: Novel polymorphisms in caspase-8 are associated with breast cancer risk in the California Teachers Study: a nested case control study

Authors:

Hannah L Park (hlpark@uci.edu)
Argyrios Ziogas (aziogas@uci.edu)
Jenny Chang (jichang@uci.edu)
Bhumi Desai (desaib13@gmail.com)
Leona Bessonova (leona.bessonova@live.com)
Chad Garner (cgarner@uci.edu)
Eun Jung Lee (Eunjung.Lee@med.usc.edu)
Susan L Neuhausen (sneuhausen@coh.org)
Sophia S Wang (sowang@coh.org)
Huiyin Ma (HMa@coh.org)
Jessica Clague (jclague@coh.org)
Peggy Reynolds (Peggy.Reynolds@cpic.org)
James V Lacey Jr (jlacey@coh.org)
Leslie Bernstein (LBernstein@coh.org)
Hoda Anton-Culver (hantoncu@uci.edu)

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Revision cover letter – Park et al., Novel polymorphisms in caspase-8 are associated with breast cancer risk in the California Teachers Study

In response to Reviewer Jeffrey Smith’s Report:

Thank you for your careful review of our manuscript.

Major Compulsory Revisions: Thank you for pointing out that clarification is needed about the SNP selection process. We have added details to the manuscript in the Methods, Genotyping section, lines 131-136. Patterns of LD between the significant SNP, rs2293554, and the CASP8 SNPs that were found to be associated with breast cancer in the BCAC paper are now described in the Discussion, lines 232-248, and shown in Supplementary Table 2.

We agree with Dr. Smith’s assertion that analyzing tumors by subtype of Luminal 1, Luminal B, HER2, and TN/basal is clinically preferable. We had originally intended to take this approach; however, since the earlier diagnosed cases did not have HER2 data available, some of the resultant groups would have had too small numbers of cases (38 for HER2 and 60 for TN/basal). Thus, we elected to do the analysis for ER and HER2 status as the categories, which is still a clinically relevant approach since selective estrogen receptor modulators
(SERMs) and aromatase inhibitors are known to be only effective in preventing ER+ breast cancers, and while drugs like Herceptin have not been approved for chemoprevention, they are used to target HER2+ breast cancers regardless of hormone receptor status. Thus, there is still clinical relevance in studying risk for breast cancers that are ER+ v. ER- and HER2+ v. HER2- as single marker subgroups.

We agree that the # of cases in each subgroup would be helpful. We have included this information along with the genotype distributions for each subgroup in the revised Supplementary Table 1. Also, we have added clarification to the Methods section that the rationale of potential increased subgroup homogeneity to improve power is the premise of the study (lines 161-163).

Discretionary Revisions: We agree that presentation of genotype distributions for each subgroup would be useful. We have included this information in the revised Supplementary Table 1.

Since only 3 pairs of SNPs on our panel had an r2>0.4, we think it is sufficient to describe these in the text as it appears currently.

Minor Essential Revision:
Thank you for your suggested edits to present the findings in a more appropriate, conservative manner. The word “nominally” was added as suggested (lines 205-206). In addition, in the first paragraph of the discussion, we have added a statement about the possibility that our observation at rs2293554 may be due to chance, lines 227-230.

Discretionary Revision:
We were able to obtain HER2-specific data from BCAC through personal email communication with the data manager and included a comparison in the discussion section as well as LD data between rs2293554 and SNPs identified in the Lin et al. BCAC paper to be associated with breast cancer risk (lines 234-247).

Minor Essential Revision: We have removed the part of the 2nd sentence of the abstract about side effects, as suggested.

Discretionary Revision: In the introduction, we make mention of the 3 CASP8 SNPs that have been identified to be associated with breast cancer in different BCAC studies. rs1045485 is the only one that affects the protein. rs1045485 encodes an amino acid residue that is localized on the external surface of pro-caspase 8 and that could affect the processing of pro-caspase 8 or caspase-8 interaction with other apoptosis regulators. However, until this is confirmed, the effect of this SNP on caspase 8 activity remains elusive (Rihani et al, 2013). The other 2 SNPs are intronic and their effects on CASP8 activity are unknown.

Minor Essential Revision: All of our cases were determined from data from the California Cancer Registry, which is based on pathology reports. We have clarified this in the Methods section (lines 110-112).
The term is used to describe the HapMap population that was used to to the LD analysis.

The SNPs on our panel were chosen because they were in their own LD bin. However, it is not known why they were chosen over presumably others that may have been present in the same bin. Most of them are intronic, with the exception of rs1045485. For most of the SNPs, they were the only ones in their LD bin (r^2#0.8). The SNPs on our panel are each in their own LD bin except two of them are in strong LD with each other according to the HaPMap-CEU. These two SNPs and another SNP on the panel are in strong LD with two other SNPs on CASP8 (not on our panel) that are missense. Functions of those other SNPs are not known. This has been added to the paragraph about potential function (lines 278-283).

In response to Reviewer David Goldgar’s Report:

Thank you for your careful review of our manuscript.

Major Compulsory Revisions

1. Thank you for encouraging us to look into the BCAC/iCOGS data. Upon inquiring with the BCAC data manager, Manjeet K. Bolla, and statistician, Kyriaki Michailidou, we were informed that rs2293554 was analyzed in their study and was not found to be associated with either overall breast cancer risk or HER2+ breast cancer risk. This discussion has been added to the manuscript. In addition, we discuss LD between rs2293554 and significant CASP8 SNPs in the BCAC paper (lines 231-247).

Minor Essential Revisions

1. We reported the unadjusted and adjusted ORs for all in a revised Table 2.
2. We acknowledge that the OR of almost 2 is quite large and added a comment on this in the discussion, lines 224-227. The SNPs have been reordered by chromosomal location in all tables.
3. Yes, there was limited overlap of samples between our study and the BCAC study (57 cases and 49 controls). This has been added to the discussion, lines 231-233.
4. Genotype distributions by subtype have been added to the revised Supplementary Table 1.

Discretionary Revisions

1. We appreciate the suggestion to impute HER2 status but we elected not to do this at this time.
2. According to University of Washington’s GVS, the r^2 between rs1861270 and rs2293554 is 0.033. We did not include this data point in the paper. However, if the reviewer thinks it is important to include, we can do so.