Author's response to reviews

Title: Mammographic density and epithelial histopathologic markers

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Author's response to reviews: see over
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Re: Mammographic density and epithelial histopathologic markers

Dear Editor:

Thanks for the kind review of our manuscript and the insightful comments from the reviewers. In response, we made the following changes to the manuscript and marked them in grey:

**Reviewer 1**

1. The main issue I have with this paper is that the authors have written their results and conclusions as though the findings are statistically significant, when they are not. …In my interpretation, there are no associations between histopathologic markers and breast density. The few associations that are significant are likely the result of chance given the large number of associations the authors have evaluated. I realize the authors are trying to generate hypotheses to be tested, but they should make substantial revisions to their abstract, results, discussion, and conclusions to reflect the true message of the paper. *We agree and modified abstract, results, discussion, and conclusions substantially.*

2. Methods: on page 6, the authors state that 118 breast tissue samples were recategorized as benign or malignant. Why? On what basis did the authors recategorize tissue samples? This raises another question, what was the timing of the collection of tumor samples relative to each woman’s diagnosis? Were they from prior benign biopsies? … If some of these are from the breast cancer diagnosis and were recategorized as benign based on cell composition, I would consider excluding these as the marker levels in this tissue may be different than those in tissue from women who have never been diagnosed with breast cancer. *This study is based solely on tumor blocks from breast cancer patients obtained after diagnosis. No prior benign biopsies were available for any subject. We clarified this in the methods. The pathologist performed the recategorization on the tissue cores after the technician had placed the 4 presumed benign and the 4 presumed malignant cores. Apparently, for these 118 cores, the area where the technician had taken the core was not what was intended. Thus, the pathologist corrected the presumed behavior (benign vs. malignant) of these tissue samples.*

3. Methods: The authors state that mammograms were scanned at a resolution of 98 pixels per inch. Was this for both small and large films? What conversion factor did you use to convert pixels to cm-squared? The authors state that the CC view closest to, but before breast cancer diagnoses was used. Can you provide more information on the timing of exams relative to 1) diagnosis, and 2) collection of the tissue sample? It would be helpful to see the median and range of dates for each of these. *All mammograms were scanned at the same resolution. We scan at 260 µm (≈97.7 dpi), so for the conversion, we divide the pixels by 1479, i.e., one cm corresponds to 38.46 pixels and 1 cm² to 1479 pixels. The conversion factor is 0.000676. As to the dates, we added the mean time between mammogram and breast cancer diagnosis; it is 10 months. Since all samples were obtained from the tumor blocks at breast cancer surgery, we only know the date of diagnosis. There is no separate date for collection of the tissue sample.*

4. Methods: the authors have adjusted for 9 covariates in their models, which is a lot given the small sample sizes (particularly for the stratified analyses). I know all of these are associated with mammographic density, but are they all associated with the histopathologic markers? Were the adjusted and unadjusted results markedly different to warrant including these in the model? *No, not all covariates were associated with all histopathologic markers, some only with a few. However, for consistency we prefer to use the same covariates in all models.*

5. Methods, page 8: The authors state, “Given the small sample sizes, we evaluated the mammographic difference between groups based on their size and consistency when the results were not statistically significant.” What does this mean? The authors looked for patterns in the results? I
think this is reasonable, but only when differences are large (>10% percent density) and the results border on statistical significance. Otherwise, there is no basis for the conclusions other than chance. *We agree that the differences were very small and most likely due to chance. As described above, the text was modified accordingly and we also removed the sentence about the consistency.*

6. Table 1: please include the histopathologic markers in this table. Were there differences in marker results in the TMA sample compared to the original study? *Unfortunately, this was not possible. For the 448 participants of the original case-control study who were not part of the TMA study (N =159), we did not have tumor blocks and no histopathologic marker information is available.*

7. Conclusions: Please be more specific about the hypotheses you have generated for future investigations. Few results were consistent or significant in this study, and I think the conclusions need to be more focused in terms of what might be meaningful to examine in the future. *As suggested, we now focus on two topics that may be of interest in future investigations.*

Minor essential revisions:
1. Abstract: specify that the study population includes pre- and post-menopausal women. *We did.*
2. Abstract: the 2nd sentence of the methods states that density was assessed from prediagnostic mammograms. Were these all screening mammograms prior to diagnosis (and not diagnostic)? *Unfortunately, we do not know the purpose of the mammograms, they could be screening or diagnostic mammograms; we only know the date of the mammograms.*
3. Abstract: In the methods, please provide the sample size and explain that the sample includes benign tissue taken prior to diagnosis for women with breast cancer. This is not clear in the abstract until one reads the methods of the paper. *As requested, we added the sample size. We also clarified that the benign tissue was taken from the surgery specimen after diagnosis not from prior biopsies.*
4. Abstract: please define TMAs. *Thank you, we did.*
5. Methods: on p 5 the authors state that 1,773 tissue samples were available for analysis. How many women does this include? *For 268 women, at least one malignant or benign sample was available.*
6. Discussion: page 12, the authors stated that they identified benign specimens for only 120 out of 279 subjects? I thought there were 159 samples in the study? *We apologize, we corrected the typo.*
7. Table 1: The numbers for age at first live birth, <30 years, cannot be right. Perhaps the columns were switched? *Thanks for detecting this error; it was corrected.*

Discretionary revisions:
1. Table 2 does not add much to the results. I would replace this with the information provided in the first paragraph of the results on the differences in density by race. *We agree and removed it, however, without adding another table. The ethnic differences have been published repeatedly.*
2. Figure 1, I would delete as this does not provide useful information to the reader. *We agree, it does not add much. Figure 1 was removed.*
3. Why would results for ER-alpha and ER-beta differ? Can the authors provide any explanations for this? *Yes, we added a sentence to the introduction about the possible differences in the receptors.*
Reviewer 2
Introduction: The first sentence should state that the relative risk of highest density is compared to the lowest density group. We did as recommended.

Line 4: Need to be more specific regarding how such knowledge may contribute to prevention. That is a good point; we added some thoughts to the sentence.

Methods: It is not clear if the density measurements from the parent study were used or were obtained for this study specifically? All images were assessed for densities as part of the parent study; no new measurements were obtained for the current study. We clarified the text accordingly.

Results:
2nd paragraph: Using the terms such as “substantial differences were seen” seem misleading. Authors should try to use terms as although numerical differences were observed, they were not statistically significant. As described above, we changed the wording in the results and the discussion.

The statement that most associations were not statistically significant implies that some were significant. Is this true? Yes, two associations in Caucasian women were, but we agree that these are probably chance findings. The wording throughout the manuscript was changed.

The 2nd paragraph gives the impression that these are true associations. Given the lack of statistical significance, as mentioned above, it should be stated that although there are numerical differences, they are not significant. The language was changed throughout the results section.

3rd paragraph: Is the breast size just a function of race? Yes, it is strongly related, but not all Caucasians have large breasts and not all Japanese have small breasts.

Discussion:
What markers the authors believe should be further studied as risk/response biomarkers. Is PR expression, given the numerical association with breast density a reasonable candidate? What markers should not be pursued? HER 2 or Ki67, given the small number of women with positive staining? Further studies with PR to understand the estrogenic effects and with Ki67 and other markers to assess proliferation may be useful; we modified this part of the discussion.

I am not sure what the value of Fig 1 is? May be removed. We removed Figure 1.

5: Conclusions need to be rewritten; it has to be more specific. Is TMA feasible or not for future studies? What hypotheses could be generated? Higher PR expression was not significant and no positive conclusion can be made such as “higher cell proliferation is present”? Is PR expression a marker of proliferation? Thanks, the revised version includes a few specific ideas for future research. As to PR, it is an indicator of estrogenic effects in breast cells not of cell proliferation.

8: Abstract needs revision: Since the results were not significant. No claims of association can be made. Only numerical differences can be stated. In the conclusion of abstract again, no claims of association can be made. Rather than blanket statements as feasibility and problems, please be more specific. Also instead of saying hypotheses have been generated, please be more specific. As suggested, the abstract was substantially reworded.

Yours sincerely,

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