Author's response to reviews

Title: Association of High Obesity with PAM50 Breast Cancer Intrinsic Subtypes and Gene Expression

Authors:

Marilyn L Kwan (Marilyn.L.Kwan@kp.org)
Candyce H Kroenke (Candyce.H.Kroenke@kp.org)
Carol Sweeney (Carol.Sweeney@hsc.utah.edu)
Philip S Bernard (Philip.Bernard@hci.utah.edu)
Erin K Weltzien (Erin.K.Weltzien@kp.org)
Adrienne Castillo (Adrienne.Castillo@kp.org)
Rachel E Factor (Rachel.Factor@path.utah.edu)
Kaylynn S Maxfield (Kaylynn.Maxfield@hsc.utah.edu)
Inge J Stijleman (Inge.Stijleman@hci.utah.edu)
Lawrence H Kushi (Larry.Kushi@kp.org)
Charles P Quesenberry (Charles.Quesenberry@kp.org)
Laurel A Habel (Laurel.Habel@kp.org)
Bette J Caan (Bette.Caan@kp.org)

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Author's response to reviews: see over
March 11, 2015

Response to Reviewer Comments: MS: 2000601663145896 - Association of High Obesity with PAM50 Breast Cancer Intrinsic Subtypes and Gene Expression

Dear BMC Cancer Editorial Board,

Please find below our Response to Reviewer Comments. If you would like further clarification or need more information, please let us know.

Thank you for your consideration of our manuscript.

Sincerely,

Marilyn L. Kwan, PhD

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Reviewer 1

Major Compulsory Revisions

A substantial concern relates to the limited sample size for some of the main and several of the stratified analyses presented in the manuscript. It is difficult to appreciate the degree of precision in the tables since no data on cell sizes are presented. For some tables, the reader has to do a bit of calculation to derive at these; in others, it is not possible to derive these, at least to this reader (i.e., no. of pre- and post-menopausal cases for each intrinsic type, related to Table 4). This reviewer is left with difficulty interpreting results for the following groups:

1. Using table 1 to derive sample sizes, it appears that findings for highly obese women appear to be based on a total of 183 cases. This number is then split to take into account the gene expression and intrinsic subtypes. These are then further split to stratify by menopausal status.

2. Pre- and post-menopausal differences for just about all comparisons, although specifically problematic for those in table 4. For example, it appears that the data in this table for basal like associations in pre-menopausal women based on about 40 cases total, distributed among 5 BMI categories. A similar problem occurs for HER2 positive cases.

3. It is nearly impossible to say anything about the underweight women. For example, in table 4, it appears that the number of basal-like underweight women category is only 2 cases. How can analyses be conducted stratifying by menopausal status? Perhaps my figures are incorrect, which is why more data are needed in the tables.
1. Given these sample size limitations, perhaps the authors should consider streamlining their analytic approach to results where precision is not a major issue.

We acknowledge the reviewer’s comments and concerns, and want to emphasize that this analysis is the first of its kind (to our knowledge) to examine obesity before breast cancer diagnosis and the likelihood of developing a specific tumor gene profile. It’s an exploratory analysis, which we now mention in the Introduction on page 5. We also state that interpretation of the menopausal results is limited in Table 4 on page 16, and acknowledge our limited sample size for the underweight group on page 19.

Minor Essential Revisions

1. The pooling of two studies that have key differences is potentially problematic. One recruited early-stage cases 15 or so years ago from a mixed Utah population (some from the cancer registry and some from KPNC) of patients enrolled within 2 years of diagnosis. The second recruited women more recently with presumably has no stage eligibility criteria (although this is not specifically noted) and enrolled within 2 months of diagnosis; importantly, this study comprised of a diverse racial/ethnic population. Given these differences and the aims of the study, investigators need to provide justification for combining the studies. At the very least, a variable for study site should be included in the multivariate models and sensitivity analyses should be conducted to see whether major findings apply to both study populations.

The Pathways and LACE studies were pooled for the PAM50 study because the overall goal was to evaluate the performance of the PAM50 assay in a population-based study where patient characteristics, treatment patterns, and time of initial follow-up varied. We now provide more details on the two cohorts, as well as a justification explaining why the cohorts were combined for this study, on page 8. As suggested by Reviewer 2 below, we also provide the results for the individual studies as new additional files. Finally, to account for underlying study differences as suggested by the reviewer, we have now adjusted for study in all main multivariate models and have updated the tables and text accordingly. Results remain consistent with what we originally reported in the first version of the manuscript.

2. Please provide more data in the tables so that the reader can easily get a sense of the cell sizes.

We’ve added more sample size information to Tables 1-4, as indicated in red.

3. A factor not related to sample size per se is the classification of Asian women into the BMI categories using the same cut-points as those for non-Asian women. Can the authors comment on the appropriateness of this approach?

The literature has suggested that the association of BMI with body composition and health outcomes may differ between Asians and Europeans. Specifically, for a given BMI, Asians generally have a higher percentage of body fat than do Europeans. Furthermore, it has been shown that Asian populations with average BMI below the standard overweight BMI cutoff point have an increased risk of
Type II diabetes and cardiovascular risk factors. As a result, it has been suggested that the BMI cutoff points for overweight and obesity should be lower for Asian populations (≥23 for overweight and ≥27.5 for obesity). However, a 2004 consensus statement from the WHO (which we cite in our paper) concluded that the available data were not sufficient to support Asian-specific BMI cutoff points, and continued to support the current WHO cutoff points for BMI classification [WHO Expert Consultation (2004) Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 363: 157-163]. Therefore, we did not apply the proposed Asian-specific cutpoints to our BMI data for analysis. Of note, we did explore the shift in numbers using the standard WHO BMI classification compared with the Asian-specific BMI classification in our n=154 Asians in the study sample (see table below). As expected under the Asian BMI classification, there is an upward shift of numbers in the overweight (27.3% to 43.5%) and obese (10.4% to 19.5%) groups with a corresponding decrease in the normal weight group (61.0% to 35.7%).

<table>
<thead>
<tr>
<th>WHO BMI Classification</th>
<th>N (%)</th>
<th>Asian BMI Classification</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18.5 (underweight)</td>
<td>2 (1.3)</td>
<td>&lt;18.5 (underweight)</td>
<td>2 (1.3)</td>
</tr>
<tr>
<td>18.5 - 24.9 (normal weight)</td>
<td>94 (61.0)</td>
<td>18.5 – 22.9 (normal weight)</td>
<td>55 (35.7)</td>
</tr>
<tr>
<td>25.0 – 29.9 (overweight)</td>
<td>42 (27.3)</td>
<td>23.0 – 27.4 (overweight)</td>
<td>67 (43.5)</td>
</tr>
<tr>
<td>≥30.0 (obese)</td>
<td>16 (10.4)</td>
<td>≥27.5 (obese)</td>
<td>30 (19.5)</td>
</tr>
<tr>
<td>Total</td>
<td>154</td>
<td>Total</td>
<td>154</td>
</tr>
</tbody>
</table>

Reviewer 2

Minor Essential Revisions

1. Particularly relevant to this study are some of the biases introduced by pooling the LACE and Pathway studies. The authors acknowledge in their discussion that including women enrolled in the LACE study (average enrollment 2 years post diagnosis) could potentially bias against the inclusion of more aggressive tumours. Tumours with higher proliferation rates (and lower ER expression) are more likely to recur early and be excluded from the LACE study. Equally important, however, is data from the authors’ prior study [ref 46] that show that there were significantly more obese women in the Pathway study versus the LACE study. If there is also a disproportionate number of highly obese women represented in the Pathways study (average enrollment 2 months post diagnosis), there is certainly a concern that this may influence the proportion of tumours with lower ESR1 and higher proliferation gene expression in the final analysis as these tumours are more likely to be captured in the Pathway versus LACE study. The authors should address these confounding biases more directly and consider showing the data and analyses from the individual study cohorts to demonstrate their findings of lower ESR1 expression (and higher proliferation gene expression) in the highly obese is not a spurious result of combining these two particular study samples. Of note, the authors have clearly considered and performed this analysis and do state that restricting the analyses to the individual trial cohorts resulted in findings "similar" to the combined data. These biases are central to the study findings, however, and deserve to be addressed with greater detail within the manuscript.
We have now added two additional files showing the study results restricted to the LACE (Additional file 1) and Pathways (Additional file 2) cohorts. To note, due to sample size limitations, we could not run the multinomial logistic regression models stratified by menopausal status, thus only results for the overall cohort are shown (Table 3). While there are more obese women in the Pathways cohort (34.1%) compared with the LACE cohort (24.5%) as presented in our previous paper [Caan BJ, et al. Intrinsic subtypes from the PAM50 gene expression assay in a population-based breast cancer survivor cohort: prognostication of short- and long-term outcomes. CEBP 23: 725-734], the gene expression results for proliferation remain similar to those in the combined cohort when restricting the analyses to the individual cohorts (see Table 2 of each Additional file). Specifically, highly obese women had tumors with higher proliferation expression compared with normal weight women. However, while both cohorts showed trends of lower ESR1 expression among the highly obese, the associations were non-significant in LACE. Finally, as suggested by Reviewer 1, Minor Essential Revisions Comment 1, we have adjusted for study in all of the main multivariate models, and results remain consistent with what we originally reported in the first version of the manuscript.

2. The BMI for this study was computed from self-reported weight and height data. The biases around self-reporting of these variables and resultant skewing of obesity-related epidemiological studies are well known and studied and should be discussed as a limitation of the study.

We have now included a statement on the limitation of self-reported weight and height data in the Discussion on page 19.

Discretionary Revisions

1. I would recommend that the authors change the wording in the methods-study population section, 1st paragraph describing entry criteria into the LACE trial: “no breast cancer recurrence or other cancer diagnosis within five years of enrollment”. This can potentially be misinterpreted as meaning no breast cancer recurrence within 5 years of enrollment.

We have revised the wording to “…no prior history of breast cancer or other cancer in the last 5 years.”
Reviewer 3

Minor essential revisions

1. It's not obvious from the abstract that this is a cross-sectional study. It should be stated up front.

   We now mention that this is a cross-sectional study in the Methods of the abstract.

2. The authors should briefly discuss the limitations of self-reported height and weight perhaps particularly among women who know they have breast cancer and the potential effect of measurement error.

   See response to Reviewer 2, Comment 2 above.

3. Why didn't you adjust for smoking? While associations are weak with breast cancer overall, there are potential associations with subtypes and smoking is clearly associated with BMI.

   We did not adjust for smoking because smoking history at breast cancer diagnosis (never, past, and current) was not associated with BMI in our study population in univariate analysis (Table 1, p value = 0.65).

4. What is the parent study (p. 7)? LACE? Pathways?

   We now clarify that the parent study is the combined study populations of LACE and Pathways (n=4,307).

5. The authors should provide some interpretation of the magnitude of the associations in the results section instead of just higher or lower.

   We now provide interpretation of the magnitude of the associations for Tables 2 and 3 in the Results on pages 13-15. For Table 2, we include the mean values of unadjusted gene expression levels when describing the results. For Table 3 (multivariate linear regression models), we discuss the magnitude of the adjusted mean differences (beta coefficients) in gene expression levels in the BMI categories relative to the normal weight group using standard deviation units. The mean differences were transformed into S.D. units by taking the value and dividing by the S.D. for each gene in the overall, premenopausal, and postmenopausal study populations as needed. Finally, to further aid interpretation for the reader, we revised the title and columns of Table 3 to reflect adjusted mean differences in gene expression levels.

6. Please clarify how BMI was ascertained and timing—were women asked to report their weight at a reference date? Were they asked to report their current weight? It’s not clear from the methods and the stated range of BMI being from '12 months prior to two months after diagnosis' doesn’t match with LACE participant enrollment on average 2 years after diagnosis.

   We have now clarified on page 8 that LACE women were asked to self-report their weight and height at 12 months before breast cancer diagnosis on the baseline questionnaire, which was on
average 2 years post-diagnosis, and Pathways women were asked to self-report their weight and height at the time of the baseline interview, which was on average 2 months after breast cancer diagnosis.

Discretionary revisions

1. Not sure of the utility of the clinical tissue markers section in the methods. I can’t where any data about ER/PR/HER2 from IHC are referenced or used in the analysis or appear in the results.

   We have removed this section from the manuscript.

Editorial Edits

1. Please re-phrase your Competing Interests section to state clearly that P.S.B. is named on the patent for PAM50 which is licenced to Bioclassifier LLC.

   We have edited the Competing Interests section as specified below:

   “P.S.B. is named on the patent for PAM50 which is licensed to Bioclassifier LLC. The other authors declare that they have no conflict of interest.”

2. Requesting Ethics statement

   We have edited our Ethics statement to comply with journal requirements as specified below:

   “Participants provided informed consent under human subjects’ protocols approved by the institutional review boards (IRB) at KPNC (CN-98BCaan-04-H) and the University of Utah (IRB_00038002). All human subjects’ research carried out in this study was in compliance with the Helsinki declaration (http://www.wma.net/en/30publications/10policies/b3/index.html).