Author's response to reviews

Title: Genetic analysis of the vitamin D receptor gene in two epithelial cancers: melanoma and breast cancer

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Version: 2 Date: 26 November 2008

Author's response to reviews:

Dear Claudia:

Thank you for giving us the opportunity to submit a revised version of our manuscript (MS: 1386456358229273) entitled: Genetic analysis of the vitamin D receptor gene in two epithelial cancers: melanoma and breast cancer. We would also like to thank the reviewers for their comments and suggestions. We have responded to each comment in turn below and have revised the manuscript accordingly, as indicated (changes made are highlighted in yellow). We have included another author J.A.Avilés, we apologise for this oversight.

Sincerely,
Gloria Ribas

Reviewer's report

Title: Genetic analysis of the vitamin D receptor gene in two epithelial cancers: melanoma and breast cancer

Version: 1 Date: 17 November 2008
Reviewer: Victoria Stevens

Reviewer's report:
This study investigates the association of SNPs in the vitamin D receptor (VDR) gene with breast cancer and melanoma using two case-control populations from Spain. One significant association was found for breast cancer while none were found for melanoma. Investigations into associations with clinical, personal and tumor characteristics revealed a few significant findings of questionable meaning. In general, this study was well conducted but the results are somewhat over interpreted. Only one SNP was significantly associated with breast cancer in the multivariate analysis. Given the large number of comparisons done (at least 8 for Table 2 and 61 for Table 3), this result and the subgroup investigation are likely to be due to chance. Other criticisms of the manuscript are detailed below.

We have gone through the manuscript and toned down the interpretation of results.

1. The background section included before the introduction is not necessary.

We have included this section as BMC Cancer suggested in their web page: instructions for authors.

2. Similarly, the first paragraph of the results and discussion section, which provides additional introduction, is also not needed.

We have removed this paragraph from the revised version, as suggested.

3. The controls for the melanoma cases were frequency matched by sex and age. Was this done for the breast cancer cases? If not, why not?

No, the design was different. All cases and controls were women and controls were selected so that their age range was comparable to that of cases. We could not frequency match due to the larger numbers of cases. This information has been added into the methods section.

4. Supplemental table 2 is incorrectly labeled as table 1.

This error has been corrected.

5. Three of the four SNPs are commonly studied and are well known (for better or worse) by researchers in the field as Fok1, Taq1, and Bgl1. To facilitate the comparison to previous findings, these names should be used throughout the manuscript.

We have included these aliases throughout the manuscript.

6. The bold used to indicate the significant findings in Tables 2 and 3 is not evident.
We have increased the font size of these results in order to obtain a better visualization.

7. The association of rs731236 with breast cancer is not longer statistically significant after adjustment for covariates. This should be acknowledged in the text.

We have included the following paragraph in the results and discussion section:
This per-allele OR estimate was not substantially different in the multivariate analysis adjusting for age, number of live births, age at menarche, and menopause status (OR per allele=0.85, 95% CI 0.69-1.03, p=0.102).

We have also added a comment in the “further considerations” section:
The association of rs731236, TaqI and BC, was not statistically significant under a multivariate model. However, the estimated relative risk did not change substantially, indicating that the increase in p-value was due to the reduced sample size (with available covariate data), rather than due to confounding.

8. It is not clear whether the ORs shown in Table 3 are the result of adjusted or unadjusted analyses/ This should be clarified. Additionally, what the reference group for each of these analyses is should be specified.

We have specified in the footnotes to the table that they are unadjusted p-values. In the case of the reference groups used to estimate ORs, they are as specified in the methods section:
Eye colour (blue/green versus brown), hair colour (blond/red versus brown/black), skin colour (fair versus brown), number of nevi (# 50 versus < 50), presence of lentigines (yes versus no) and childhood sunburn (yes versus no) were used as the outcome variables for MM.
Among BC cases only, the presence of metastatic disease at diagnosis (yes versus no), tumour histology (invasive versus in situ), tumour grade (grade > 1 versus grade 1), tumour size (> 2 cm versus # 2 cm), nodal involvement (yes versus no), estrogen receptor status (positive versus negative) and progesterone receptor status (positive versus negative), were used in the analysis. For MM cases-only analyses, the prior diagnosis of MM (yes versus no), phototype (I/II versus III/IV), tumour location (head/neck/trunk versus extremities) and tumour depth (T2/T3/T4 versus T0/T1) were considered as the outcome variables.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.

Reviewer’s report
Title: Genetic analysis of the vitamin D receptor gene in two epithelial cancers: melanoma and breast cancer
Version: 1 Date: 19 November 2008
Reviewer: Karen Curtin
Reviewer’s report:
Discretionary revisions
1. Background paragraph, sentence 5: Remove “, for the first time,” and simply state: “In this novel study, we investigated the role of polymorphisms in VDR...”.

We have edited the text as suggested.

2. Introduction, 3rd paragraph, 2nd sentence construction: insert commas before and after the phrase “sporadic BC and MM”.

We have edited the text as suggested.

3. Materials and methods, SNP selection paragraph, last sentence: Based on the references provided, clarify that the two coding SNPs selected have been reported to be associated with breast cancer.

We have edited the text as suggested.

4. Table 2 footnotes: remove quotation remarks around adjustment variables.

We have edited the text as suggested.

Minor essential revisions
1. Background paragraph, 3rd sentence (“It is of general interest the study of the most characterised variants in VDR...) should be revised for proper English grammar and syntax.

This has been corrected.

2. Introduction, 2nd paragraph (“...there is strong evidence suggesting...”): Provide a reference or support for your statement that the evidence is “strong.”

References of functional studies have been included in the text.

3. Introduction, 2nd paragraph (“...each finding different effects for SNPs, depending on the population analyzed.” Please clarify; do different SNPs play a role in breast cancer or melanoma, depending on the population studied? Is the same SNP protective in one population, but associated with increased risk in
another population?

Each SNP may play a different role depending on exposure or environmental factors. In the case of melanoma, population background is important, especially if we take into account skin colour differences and UV exposure. UV exposure is the main exogenous agent in melanoma aetiology, but considering that UV exposure is necessary for the endogenous synthesis of vitamin D, the effect of one SNP may be modulated by several external factors.

We have added the following comment in the introduction section:
Each finding different effects for SNPs, depending on the population analyzed and environmental factors acting upon them.

4. Introduction, 3rd paragraph: You state that it is of interest to study VDR variants in southern European countries where sun exposure is typically higher. Please clarify the comparison; typically higher compared to northern European countries that have been widely studied (if this is the case)? It might be useful to provide support for your statement, for example the average UV index or latitude in Madrid compared to one or two other cities of the Caucasian populations you reference in the previous paragraph.

We have modified the text to clarify that is typically higher in comparison with northern European countries.

5. Materials and methods, study subjects, etc. paragraph: For the BC and MM studies, please include the time period (e.g. year, month) over which the consecutive cases were recruited. What was the cooperation or participation rate for cases in each study?

Precise data on participation rates were not collected. However, our experience in the clinic at the CNIO is that response rates are very high in Spain (around 90%).

We have included the time period for both studies.

6. Materials and methods: Were the controls in the BC study frequency matched to cases by age? If so, please state; if not, note that BC controls were not frequency matched to cases.

No. All cases and controls were women and controls were selected so that their age range was comparable to that of cases. We could not frequency match due to the larger numbers of cases. We have modified the text as suggested.

7. Materials and methods, statistical analysis, paragraph 2: remove comma after MM in: ...”and MM, (eye colour, hair colour, ...).

We have edited the text as suggested.
8. Results and Discussion, association of VDR rs731276 and rs2228570 polymorphisms with cancer risk, 4th paragraph, 1st sentence: correct the typo ("we did not observe" rather than "we did no observe"). Remove the paragraph indentation before the final sentence in this section, include “We did not observe evidence of association with any other SNP in BC or MM.” should be included as the last sentence of the previous paragraph, rather than a one-sentence paragraph.

We have edited the text as suggested.

9. Further considerations paragraph: It should be mentioned that the controls represent a ‘convenience’ sample and were not population based.

We have acknowledged this in the further considerations section, by including the following sentence:
Controls participated on a volunteer basis which may have introduced some selection bias. However, the fact that they were frequency matched to cases on age and sex for melanoma and that breast cancer controls were selected so that their age range was comparable to that of cases and that the variable of primary interest was genetic would have kept such bias to a minimum.

Major compulsory revisions
None.
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests.