Author's response to reviews

Title: Nuclear Receptor Coregulator SNP Discovery and Impact on Breast Cancer Risk

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Author's response to reviews: see over
10/06/2009

Regarding: **MS ID#: 1617789192294835**  
**MS TITLE:** Nuclear Receptor Coregulator SNP Discovery and Impact on Breast Cancer Risk

Dear Editor,

We are submitting a revised version of our manuscript entitled Nuclear Receptor Coregulator SNP Discovery and Impact on Breast Cancer Risk (1617789192294835), originally reviewed in September 2009 (Editor: Dr. Diana Marshall).

We would first like to thank the reviewers for their time and effort for their comments, feedback, and a very favorable review.

A statement regarding informed consent has been added per the specific request from the editor.

Below we address the comments from the reviewers in a point-by-point reply:

Reviewer 1:

1) “Although the authors mentioned Hardy-Weinberg equilibrium tests in the Statistical Analysis, there is no description (of Hardy-Weinberg calculations) on the results in the results/discussion.”

We have added a statement regarding the description of HWE calculation in the results/discussion part: “The genotyping results were in Hardy–Weinberg equilibrium in controls for all SNPs investigated (\(P = 0.309\) for rs1804645; \(P = 0.112\) for rs6094752; \(P = 0.058\) for rs2230782; \(P = 0.067\) for rs2076546; \(P = 0.140\) for rs2229840)”.

2) “Please describe the source of controls. Were they inhabitants, health checkup examinees, or patients without cancer at some hospitals? How about their age range?”

The Methods section was updated with the following information: The control population included healthy and unrelated female blood donors collected by the Institute of Transfusion Medicine and Immunology (Mannheim), sharing the ethnic background and sex with the breast cancer patients. The age distribution in the controls and cases was similar (controls: mean age 45.6 years, median age 46 years, age range from 19 to 68 years old; cases: mean age 45.1 years, median age 45 years, age range from 18 to 87 years old). According to the German guidelines for blood donation, all blood donors were examined by a standard questionnaire and gave their informed consent. They were randomly selected during the years 2004–2007 for this study and no further inclusion criteria were applied during
recruitment. The study was approved by the Ethics Committee of the University of Heidelberg (Heidelberg, Germany).

3) “The authors described in abstract ‘1,509 matched controls’, and ‘controls were taken from the same gender, geographic area, and ethnic background (Caucasian/German) as the cases’. In an epidemiological sense, the selection of controls from the same population does not mean “matching”. Please, delete the “matched” in Abstract. Matching is the technique to make the distribution of the selected matching factor among controls similar to that among cases. There are two methods; individual and frequency matching.”
We agree with this comment and have made the necessary change.

4) “Please, add the full spelling for MAF at the first appearance in the text.”
We agree with this comment and have made the necessary change.

5) “Please, replace ‘NCOA3’ in results/discussion and Table 5 with SRC-3, because the mixture of the different names for one gene makes the reader confused. The explanation of SRC-3/NCoA3/AIB1 in Background is, of course, essential.”
We agree with this comment and have made the necessary change.

6) “In line 19 of page 12, ‘95%CI=0.041, Table 3’ is ‘95%CI=0.041, Table 2.’”
We agree with this comment and have made the necessary change.

7) “In Table 2, ‘1.00’ for OR of SRC-1 should be ‘1’.”
We agree with this comment and have made the necessary change. Also changed in tables 3 & 4.

8) “In Table 3, ‘0.48’ for P should have the third decimal place, like the other P values.”
We agree with this comment and have made the necessary change.

9) “If the ORs are not adjusted for age, please describe that they are crude OR. If the adjustment was conducted for some factors, indicate it in the text and/or Tables.”
We added a statement regarding the ORs: “Crude odds ratios (ORs), 95% confidence intervals (95% CIs) and P values were computed by unconditional logistic regression using a tool offered by the Institute of Human Genetics, Technical University Munich, Germany (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl)”.

Reviewer 2:

1) “Please check the claimed association for rs1804645 with bilaterality of breast cancer. There is 1 TT genotype in 113 bilateral BC cases, and 0 in 1509 controls, so the p value cannot be 0.000025. Similarly, for TT+CT genotypes the frequency of at-risk variants in bilateral BC is 7/113 (6.2%) and in controls is 77/1509 (5.1%). This cannot result in odds ratio 40.00 and p value 0.00026. Perhaps, all other calculations have to be checked as well.”
We agree that these P-values results are weird and have therefore decided not to give any P-values, confidence intervals and Odds ratios if the frequency is 0 in controls. Regarding the
second part of this comment, we agree that there was a mistake. We intended to compare [TT] vs [CC + CT]. However, also in this comparison, we have the problem of a frequency of 0 in controls. Thus, we decided to show the result of the comparison [CT + TT] vs [CC], giving no significant result at all (Table 3). We have re-checked the calculations and this was the only mistake.

2) **All examined SNPs have to be explicitly listed in the abstract (otherwise the reader gets impression that all SNPs with MAF > 5% have been analyzed.**”

We agree with this comment and have made the necessary change.

3) **“Higher number of common SNPs per gene in Blacks versus Whites deserves discussion, with the reference on other studies analyzing SNP patterns in Africans versus Europeans.”**

The SNP@Ethnos database provides clear data regarding this statement in the Yoruban HapMap population and is likely due to population bottlenecks in the history of non-Yoruban populations. The higher number of population specific SNPs in African Americans we observed is likely due to this. This citation and a statement summarizing this were added to the manuscript.

4) **“It would be helpful to repeat the criteria for cases selection in the ‘Materials and methods’, despite this information is available in previous publications of the authors.”**

We have added the following selection criteria: Familial cases were identified based on (A1) families with two or more breast cancer cases including at least two cases with onset below the age of 50 years; (A2) families with at least one male breast cancer case; (B) families with at least one breast cancer and one ovarian cancer case; (C) families with at least two breast cancer cases including one case diagnosed before the age of 50 years; (D) families with at least two breast cancer cases diagnosed after the age of 50 years; (E) single cases of breast cancer with age of diagnosis before 35 years.

In summary, we have addressed the comments raised by the reviewers, and we strongly feel that these changes have resulted in a significantly improved manuscript. We hope that the manuscript is now acceptable for publication.

Thank you very much for your time.

Sincerely

[Signature]

Dr Oesterreich