Author's response to reviews

Title: A Polymorphism in the Base Excision Repair Gene PARP2 is Associated with Differential Prognosis by Chemotherapy among Postmenopausal Breast Cancer Patients

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Re-submission of a Research Article

MS 1466237929174592 - A Polymorphism in the Base Excision Repair Gene PARP2 is Associated with Differential Prognosis by Chemotherapy among Breast Cancer Patients

Dear Editors,

We herewith resubmit our manuscript "A Polymorphism in the Base Excision Repair Gene PARP2 is Associated with Differential Prognosis by Chemotherapy among Breast Cancer Patients" for consideration for publication in “BMC Cancer”.

We have appreciated the valuable comments of the referees and revised our manuscript accordingly. Mainly, we performed and included a meta-analysis across BCAC sites to assess study heterogeneity in more detail for the PARP2 SNP and the XRCC1 SNPs. We also added a paragraph discussing the differing results we obtained for the XRCC1 SNPs (see our point by point response attached).

This version has been approved by all authors. In addition, all authors declare that they have no conflict of interest in connection with this paper.

Please find our detailed point-by-point response attached to this letter. We submit a copy of the revised manuscript where the revisions are marked in red.

We are looking forward to receive your response.

With kind regards,

PD Dr. Odilia Popanda
Point to point response to referees

Referee 1

This manuscript has the undoubted merit to systematically present a large amount of data. Furthermore statistical analyses are well described. However it would be better to soften the conclusions and to discuss more thoroughly the possible involvement of PARP2 SNP in protein function also reporting literature data (page 11 lines 298-303).

Response: Although, only the PARP2 SNP is significantly related to survival in our analyses, there are further SNPs with similar modulating effects reported in the literature. We softened our statement in the Abstract, Conclusions (page 4, line 126), and included the phrase “together with other genetic variants”.

Regarding a possible functional impact of the PARP2 rs878156 SNP, no data have been published yet. It is however conceivable that the SNP affects splicing and changes the amino acid sequence of PARP2. Such changes are highly prone to affect the cellular function or level of PARP2. There are some hints available from the UCSC genome browser but the differentially spliced mRNA variant has still to be shown experimentally. To clarify this point we have modified the discussion on page 11, line 300-315, and included an additional reference. It reads now:

“rs878156 is an intragenic SNP in PARP2 (minor allele frequency of about 10%) located 10 base pairs distal from an intron-exon boundary without reported functional impact. Recent research showed that intragenic SNPs which are located even up to 1000 bp away from the intron-exon boundary can still affect splicing of the RNA transcript thus modifying protein levels or function [31, 32]. Similar effects are also conceivable for rs878156. This assumption is supported by an increased DNase I sensitivity, high sequence conservation of the SNP region and additional spliced ESTs indicated in the UCSC genome browser (https://genome-euro.ucsc.edu, hg19) but the altered transcript has still to be confirmed experimentally. Regarding PARP2 function, it is catalysing, together with PARP1, the poly(ADP-ribosyl)ation of various proteins involved in genome surveillance, especially base excision repair proteins, histones and transcription factors, and in this way modulates the activity of these proteins. Both PARP proteins are induced by DNA-strand interruptions but act on different lesions such as PARP1 on single-strand breaks or PARP2 on gaps and flap structures [33]. PARP proteins share considerable similarity in the catalytic domain but have different DNA binding domains [34, 33]. There are several inhibitors available affecting both enzymes and some of them are already used in tumour therapy with promising results [35].”
Minor comments

Page 5, line 148: it is hazardous to deal with “tumour elimination”

Response: Text was changed in “…is one of the crucial determinants of tumour chemotherapy”.

Figure 1: in the last box of "patients excluded" what does it mean “previous non-tumour”?

Response: We have corrected the typo and the text in Figure 1 was now changed to “previous non-breast tumour”

Referee 2

1. Is the question well defined? Yes.

2. Are the methods appropriate and well described? Yes, with a few minor issues. First, the abstract describes a multiplicative interaction term, and implies that the P-value is from the Wald test, but the methods section says that the likelihood ratio test was used. This discrepancy should be clarified.

Response: The referee is right and we corrected this point in the abstract, results (page 9, line 252 and ff) and tables 2 and 3 indicating throughout that the likelihood ratio test was used.

The meta-analysis of the MARIE results and BCAC results is appropriate, however, the BCAC results seem to have been pooled, and adjusted for study site, rather than a meta-analysis across available BCAC sites, which would make more sense.

Response: The referee is right that a pooled analysis was performed in BCAC because individual data from studies were harmonized and available. By stratifying by study in the Cox regression models, we allow for differences in the underlying function across studies, as in the meta-analysis approach. We additionally performed a meta-analysis across BCAC sites to assess study heterogeneity, which indicated very similar results as the pooled analysis (as expected) and revealed no study heterogeneity. These results are presented in supplementary figures 2 and 3 and in results (page 9, last paragraph and 10, first paragraph).

Also, I will note that the selection of confounders would be better if a 10% change in estimate was evaluated, rather than an automated procedure, such as backward selection, but I recognize that this preference may differ.

Response: We acknowledge the remark, but we prefer to use the commonly used backward selection procedure.

3. Are the data sound? Yes.
4. Are the tables and figures appropriate/acceptable? Yes, with a few minor issues. Table 1: the additional information about neoadjuvant therapy under tumor size is unclear.

Response: We clarified this point in specifying the footnote in table 1.

I prefer P-values in Table 1, but recognize that this is also personal preference.

Response: For descriptive purposes, we prefer to provide the numbers without p-values.

Figure 1 is okay, but what would be more interesting is the equivalent for the variants. If 127 were evaluated, and 14, 13, and 14 were tested for replication, how many total were tested, and then how many total replicated. By my count 4 total replicated, of which 2 are the same variant, so 3, but the paper focuses only one 1 in PARP2, and the abstract doesn’t even mention the 2 in XRCC1.

Response: The referee has correctly understood from the manuscript that 14, 13, and 14 SNPs were tested for replication with respect to differential association by any chemotherapy, anthracycline-based chemotherapy, and radiotherapy, respectively. All replication results are given in Tables 2 (chemotherapy) and 3 (anthracycline-based chemotherapy), and Supplementary Table 2 (radiotherapy) and in the results section. We therefore decided not to provide double information and left Figure 1 as is, focused on the patient population. However, we would like to emphasize that only one SNP, PARP2 rs878156 is replicated in the BCAC studies, showing a significant interaction term and the same direction of differential association by any chemotherapy as observed in the discovery study, MARIE. This SNP also showed differential association by anthracycline-based chemotherapy. Details of the meta-analyses for this SNP are given in Figure 2 and Supplementary Figure 2.

For the two XRCC1 SNPs, rs3213355 and rs3213356, there was significant differential association by any chemotherapy but not by anthracycline-based therapy in the BCAC studies. Both SNPs were in high linkage disequilibrium, therefore, we performed the meta-analysis only for rs3213356. We now report the meta-analysis of XRCC1 rs3213356 for the MARIE study and the BCAC studies according to chemotherapy (no, yes) subgroups (new Figure 3 and Supplementary Figure 3). There is significant heterogeneity between the MARIE study and the BCAC studies. The differential associations in BCAC were not in the same direction as observed in MARIE and therefore did not replicate the MARIE finding. In the MARIE study, chemotherapy was associated with a smaller hazard ratio in carriers of the variant allele, whereas in the BCAC studies with a higher HR indicating worse prognosis (see Figure 3). Therefore, we did not consider this as a replicated result and did not include it in the abstract. The significant differential association observed for XRCC1 SNPs in BCAC is therefore a new finding and needs to be replicated in independent studies. As there are several publications indicating a potential impact of XRCC1 SNPs on survival, however, we added a
paragraph in the discussion about the relevance of this gene (page 12, 2nd paragraph).

Figure 2 is not very informative. Since the paper is focused on testing interactions with variants, why not show the difference in variant effects by treatment?

Response: Figure 2 shows the association of the PARP SNP with survival according to treatment. We revised the order of presentation and the figure legend to improve clarity.

And again, how about the effects by BCAC site, then the meta across, so readers can decide if the results are robust or not?

Response: We now present both pooled analysis and meta-analyses for BCAC studies. Please refer to Supplementary Figures 2 and 3 for the meta-analyses across individual BCAC sites. See also our answer to review point 2 above.

5. Acceptable for reporting standards and data deposition? Yes.

6. Is the discussion and conclusion adequately supported? Mostly. As mentioned above, there seem to be 3 total replicated findings, but only PARP2 is discussed, not XRCC1. Plus three variants in POLB are suggestive, and since the association didn’t differ by anthracyclines or radiotherapy, what other treatments were there? Any other ideas for this potential difference?

Response: For the POLB SNPs, we also observed differential associations in BCAC, however, in the opposite direction, thus similar considerations as described for XRCC1 apply (see also response to point 4). To avoid a too strong interpretation of our results, we considered none of these associations as replicated in BCAC. They might be chance findings.

The discussion is overly focused on PARP2, and should be expanded.

Response: We focused on PARP2 because the SNP was clearly replicated, showing the same direction of SNP association by treatment in both MARIE and BCAC studies. However, we now include some more discussion on XRCC1 because despite lack of replication of the association observed in MARIE, there was consistent differential association across BCAC studies. In addition, there are several references available on the effects of XRCC1 genes on breast cancer prognosis. These references, however, concern different SNPs in the gene and different patient cohorts, and reported inconsistent findings and therefore remain inconclusive. Please read more on page 12, second paragraph.

7. Are the limitations clearly stated? Yes.

8. Appropriate acknowledgement of prior work (theirs and others)? Mostly. Additional information on prior studies that evaluated interactions between genetic variants and breast cancer treatment would be beneficial.
Response: The referee is right; there are prior studies, however, we focused the manuscript on the PARP2 SNP. In the context of the XRCC1 SNPs on page 12, we quoted some of these studies and included in the Abstract, Conclusion, line 126 and on page 13, line 358: “…rs878156 may together with other genetic variants modulate cancer specific survival in breast cancer patients depending on chemotherapy.”

9. Accurate title and abstract? Not quite. Again, the focus is on PARP2, and XRCC1 is not included or discussed. Also, results are specific to post-menopausal women, not all breast cancer cases, so this should be clarified.

Response: Due to the limitations of our replication study mentioned above we restrain from including XRCC1 in the abstract. We however included it in the discussion.

In addition we included “post-menopausal” in the title as suggested and changed it to “A Polymorphism in the Base Excision Repair Gene PARP2 is Associated with Differential Prognosis by Chemotherapy among Postmenopausal Breast Cancer Patients”

10. Acceptable writing? Yes, although at the end of the introduction, the clarification is needed that variants were tested for replication, and were not all replicated variants.

Response: We clarified the text accordingly. It reads now on page 6, line 162: “Significant associations were tested for replication in studies of the Breast Cancer Association Consortium (BCAC).”