Author's response to reviews

Title: Polymorphisms in Cyclooxygenase-2 gene and breast cancer risk in Brazilians: a case-control study

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Author's response to reviews:

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Dr Janet Hall, Editorial Board
BMC Cancer

Dear Dr. Hall,

I would like to thank you and the referees for the thorough evaluation of our manuscript “Polymorphisms in Cyclooxygenase-2 gene and breast cancer risk in Brazilians: a case-control study” (Piranda, Festa-Vasconcellos, Amaral, Bergmann and Vianna-Jorge). We have addressed all the comments and hereby present a point-by-point response. We also enclose the revised version of the manuscript, with all the changes highlighted for better identification. We hope that this improved version is now acceptable for publication on BMC Cancer.

Sincerely yours,

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Point-by-point Response to referees’ comments

Referee 1: David Cox
1. We apologize for the impression that we disregarded the large body of work published on the PTGS2 polymorphisms and their impact on cancer risk, including breast cancer. In fact, we conducted a systematic review of the literature on pubmed website (www.ncbi.nlm.nih.gov/pubmed), combining the search terms “PTGS2” or “COX-2”, “polymorphism” or “polymorphisms” and “cancer”. The search resulted in 132 papers, among which 64 used case-control design for the evaluation of the impact of PTGS2 polymorphisms on the risk of developing cancer (8 reports involving breast cancer). Most of these studies are now quoted in the manuscript (first paragraph of the Discussion) and included in the References.

The last paragraph of the discussion intended to emphasize the opportunity of combined analysis of the four most frequent polymorphisms (as a consequence of the screening strategy) rather than marginalize previous studies. However, we recognize that this intention was not clear in the text and we thank the referee for pointing that out. We rewrote the first, the second and the last paragraph of the Discussion to better acknowledge previous studies and to improve the perception on how we intend our contribution.

2. OK

3. The referee is correct that the risk association was borderline and limited to heterozygotes and it was not our intention to over-estimate this result. We have now clearly indicated this fact in the text and discussed the power limitations of our study to evaluate inheritance models (Discussion, page 12, 1st paragraph). We also clarify our interpretation that, taken together, the results appear to indicate no association between 8473TC polymorphism, either alone or in its most frequent haplotypes, and the risk of breast cancer.

4. OK

5. We evaluated that the Discussion was indeed a bit long. We excluded the evaluation of data from different types of cancer and focused the comparison of our results to studies involving breast carcinoma. We agree with the referee that screening the entire gene would be the ideal approach, considering the possibility of tagging polymorphisms. However, it would also increase cost, time and hand work. Because a vast amount of literature appears to indicate no significant association between polymorphisms in these regions and the risk of cancer, we chose to focus our analysis on the regulatory regions. We explained this rationale in the second paragraph of the Discussion and included the references to the studies of polymorphisms in exons and introns.

6. The study limitations, with regard to statistical power and to not screening the entire gene are now discussed, as explained above (items 3 and 5).

7. OK

8. The results with respect to age were removed from the abstract. The referee is correct that age is a recognized cancer risk factor and it was not our intention to explore risk limits to specific age ranges. As pointed out by Referee 2, the age difference between cases and controls was a sampling issue rather than a risk issue. The presentation of age results was also reduced (page 8, 2nd paragraph
and page 9, 1st paragraph). In relation to the P-value presented in Table IV, it was calculated using chi-square test and Pearson P-value (which is non-directional, i.e, 2 df). The information about the Pearson p-value and the chi-square test was added to the legend of Tables II, IV and V and to the section of Statistical Analysis (Materials and Methods, page 7).

9. The manuscript was revised by a native English speaker. We hope that this version is improved with regards to style and grammatical correction.

Referee 2: Thilo Dork

1. We did not perform a full screening of the gene, including the 10 exons and the intronic regions. We chose to focus on the regulatory regions of the gene because PTGS2 mRNA is very unstable, which results in COX-2 activity being mostly dependent on the protein level rather than on its catalytic function. As pointed out by Referee 1, screening the entire gene would be theoretically preferable because variants in the coding and non-coding regions could also tag other functional polymorphisms. However, previous reports involving these variants suggest no significant effect on cancer risk. We have now explained this rationale in the second paragraph of the Discussion and included the references to the studies of polymorphisms in exons and introns.

2. The referee is correct that the age difference between cases and controls was a sampling issue rather than a risk issue. We removed age results from the abstract (following the suggestion of Referee 1) and reduced the data presentation in the Results section (page 8, 2nd paragraph and page 9, 1st paragraph).

3. The distribution of color groups was not significantly different between cases and controls. This information is now stated in the text (Discussion, page 1, last paragraph).

4. We added [D´] and R2 values to Table III. The alleles -1290G, -765C and 8473C are indeed highly correlated and occur together in the fourth most frequent haplotype (Table VI). The Referee is correct that the risk association found for 8473TC polymorphism was restricted to heterozygotes. We agree that the lack of positive association for homozygotes or for 8473C carriers (in any inheritance model) as well as for 8473C-containing haplotypes weakens the evidence of 8473TC polymorphism as a cancer risk factor. We substituted the last sentence of the abstract (to avoid misleading the readers) and improved the Discussion, in order to consider the results together (page 13, 2nd paragraph). The Referee is also correct that the alleles –1195A and 8473C may segregate together (although not evident in Table III). In fact, the polymorphic allele -1195G occurs mostly alone, which constitutes the third most frequent haplotype (Table VI). This information is also given in the Results section (page 8, 2nd paragraph).

5. The Referee is correct that the risk association found for 8473TC polymorphism was borderline and restricted to heterozygotes, which limits the interpretation of its possible functional impact. This limitation is now clearly stated in the text (Discussion, page 12, 1st paragraph). There was no deviation from Hardy-Weinberg equilibrium among cases (although they do not fit the premises
for Hardy-Weinberg equilibrium). In relation to the dHPLC analysis, we agree that it might be less informative and more prone to misidentification or to sub-identification of variant polymorphisms, especially homozygotes. Nevertheless, we confirmed the genotype (by automatic sequencing) of all samples with chromatographic profile suggestive of variation. In addition, all samples with a single peak in the chromatographic profile were re-analyzed after addition of an equal amount of a wild-type control sample. This procedure is recommended to identify variant homozygotes. Finally, we obtained 100% matching results in a set of independent control analysis, using automatic sequencing (described in Materials and Methods).

6. We thank the Referee for the careful observation of Table VI. The haplotype analysis was performed using genotype information from all subjects (319 cases and 273 controls). However, in some cases, there was no complete information for the four individual genotypes (Table V indicates the total number of genotyped samples for each polymorphism). The figures in the bottom line of Table VI corresponded to the number of subjects considered for haplotype inference using Haploview software (302 cases and 264 controls). The discrepancy between the sum of individual haplotypes and the total number in the bottom line was due to the omission of a few rare haplotypes. We apologize for the inconsistencies and for the lack of appropriate explanation in the text. The information about the number of samples available for haplotype inference was added to the Results section (page 9, last paragraph). The figures in Table VI were corrected to indicate the number of haplotypes (instead of individuals) and this explanation was added to the legend. The rare haplotypes were also included.

Referee 3: James McKay

1. There were no differences in the allelic frequencies among the Brazilian color groups, either in the general population or in breast cancer patients. Likewise, the distribution of color groups did not differ between cases and controls (Table V). Nevertheless, we considered the Referee concern, and evaluated the variable “color” in a multivariate logistic regression analysis (using the Enter mode in SPSS), but it had no effect on the risk assessment of 8473TC polymorphism.

2. The analysis of LD was conducted only for the four most frequent polymorphisms. The other 5 polymorphisms have very low frequencies and were not genotyped in the general population or in the case-control set. We recognize that multiple testing may lead to spurious associations and that we could have set the level of significance at 0.05/4 (considering the polymorphisms studied) or at 0.001 (as mentioned by Referee 1 in reference to the post-genome era). We chose to show the non-corrected P-value in each independent evaluation, as it was done also for the clinical features. We discuss the limitations for data interpretation in view of the relatively small size and restricted statistical power of our study.