Author's response to reviews

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

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Author's response to reviews: see over
Dear Dr. Hall,

I would like to thank you and the referees for the further evaluation of our manuscript, now entitled “Polymorphisms in regulatory regions of 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 additional 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 version is now acceptable for publication on BMC Cancer.

Sincerely yours,

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Referee 1: David Cox

1. We rewrote the conclusion paragraph in the discussion to include the text suggested by the referee, i.e. that “variants in the promoter and 3' UTR of PTGS2 do not appear to greatly influence breast cancer risk”. We also stated that the apparent risk association found for 8473TC (rs5275) heterozygotes showed lower bound of ORs, with borderline significance. We do not agree that the analysis of haplotypic groups in Table 6 was analogous to an allelic test for the 8473TC polymorphism because the reference group was the major haplotype (i.e. composed by the four major alleles) instead of any haplotype containing the 8473T allele. Nevertheless, we agree that the association statistics for each haplotype should be presented. The results indicate that some haplotypes might affect the cancer risk, but this assumption needs further evaluation in larger studies. This comment was added to the conclusion.

Referee 2: Thilo Dork

5. We agree with the referee that any level of protein will likely be irrelevant without activity. However, the SNPs in coding regions of PTGS2 have already been shown not to interfere with protein activity (Fritsche et al., 2001; Ref. 48) or with the risk of developing cancer (see Discussion, paragraph 2). In relation to the possible regulatory effects of the SNPs on mRNA levels, Zhang et al. (2005; Ref. 10) show that the polymorphism -1195AG (rs689466) eliminates a binding site for c-MYB and leads to a 4 to 6 times reduction on the gene promoter activity (evaluated by a luciferase gene reporter system). In relation to the polymorphism -765GC (rs204127), results are conflicting: Papafili et al. (2002; Ref. 9) show a reduction in the gene promoter activity (evaluated by a luciferase gene reporter system), whereas Szczeklik et al. (2004; Ref.47) report a 10 times increase in PGE2 production by human monocytes. These studies are now referred in the Discussion (paragraph 2) to support the rationale for the preferential analysis of untranslated regions.

6. We agree with the referee that a negative result does not necessarily exclude a potential low risk. The two-fold increase was used as a first estimation in order to allow calculation of the sample size. The detection of lower risks would indeed require larger individual studies or meta-analysis. This is now stated in the Discussion (page 13, paragraph 2).
7. We thank the referee for the careful reading and we apologize for the mistake on the reference numbers (accidentally not corrected in the revised version). We have now corrected the citations.

8. We followed the referee’s suggestion and included the term REGULATORY REGIONS in the title.

9. We corrected “infinity” and substituted “comprehend” for “encompass”. The word “cycles” (or any possible mistyping of it) could not be found in the text. We hope that this version now conforms to the expected language precision.

Referee 3: James McKay

We now refer preferentially to the rs numbers in order to identify genetic variants. The other polymorphisms aliases are presented only in their first citation.