Author's response to reviews

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

Authors:

Diogo N Piranda (diogopiranda@yahoo.com.br)
Juliana S Festa-Vasconcellos (juliana.s.festa@gmail.com)
Laura M Amaral (murta.laura@gmail.com)
Anke Bergmann (abergmann@inca.gov.br)
Rosane Vianna-Jorge (farmaco@inca.gov.br)

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Author's response to reviews: see over
Dr Janet Hall, Editorial Board
BMC Cancer

Dear Dr. Hall,

I would like to thank you and the Associate Editor for the further evaluation of our manuscript, 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,

Rosane Vianna-Jorge, PharmD, PhD
Associate Professor
Institute of Biomedical Sciences – Federal University of Rio de Janeiro
Head of Research Group, Clinical Pharmacology and Pharmaceutical Assistance
Division of Pharmacology, Brazilian National Cancer Institute
farmaco@inca.gov.br
Phone: + 55 21 3233-1292    Fax : +55 21 3233-1340
1. We followed the Associate Editor’s suggestion and substituted the term ‘prevalence’ for ‘minor allele frequency’ in the Abstract and throughout the manuscript in order to avoid any possible misinterpretation. The screening of the 67 individuals was indeed performed to identify PTGS2 polymorphisms among Brazilians and to select those with minor allele frequency (MAF) of at least 10%. This information is now in the Abstract and is also described in page 6 of the manuscript (Materials and Methods – Experimental Design and Study population). We included the screening results for all the SNPs in the Abstract. We also included a mention to the linkage disequilibrium among the four most frequent SNPs and we rewrote the conclusion remark.

2. The diagnosis of breast cancer was confirmed with histopathology for all the patients. This information was added to page 7.

3. The healthy controls were all women. This information was already stated at page 7, line 4. The DNA was obtained from peripheral blood cells for all subjects (volunteers, cases and controls). The volume of blood was 3 mL. The blood was not frozen, it was kept under 4°C before DNA extraction, which was performed until 24 h after blood collection. These additional informations are now presented in the text (page 7, SNP Screening and Genotyping).

4. We agree that the genotyping description was a bit confusing. The 10 PCR reactions listed in Table 1 were used for the screening analyses, which were conducted in the 67 healthy volunteers. Three SNPs could not be identified by dHPLC and were genotyped by PCR-RFLP, both in the screening set and in the case-control study. The four SNPs with MAF >10% were further characterized in the additional sample of healthy volunteers (355). These SNPs were also selected for the case-control study and were genotyped using the same sets of primers described in Table 1. We rewrote the first paragraph of Experimental Design and Study Population and the whole section of SNP Screening and Genotyping. We hope that the methodological description is now clear enough. We added the RS numbers of the SNPs identified in each PCR reaction to Table 1. No SNP was identified using the fragments AM2 and AM4. In Tables 2 and 5, the missing data are due to lack of PCR amplification. This information was added to the Tables’ legends.
5. The first set of results (I suppose the Editor is referring to the data in the first paragraph) is indeed based on the first 67 individuals screened. We added this information (page 10, line 2).

6. We added a line for each clinical variable in the Table 4, with the number of individuals for which the information was not available.