Reviewer's report

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

Version: 3 Date: 17 May 2010

Reviewer: Thilo Dork

Reviewer's report:

The authors have significantly improved their manuscript and addressed most of the previous points. A few issues remain as detailed below.

1. Is the question posed by the authors well defined?

I think it is sufficiently well defined.

2. Are the methods appropriate and well described?

This has been improved by adding sequencing data and further dHPLC validation results.

3. Are the data sound?

This has been improved. My previous points 2,3,4 and 6 have been addressed.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?

Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data?

This has been improved, and the authors are now much more cautious with the interpretation of their borderline result on heterozygotes for T8473C. A weakness remains with the voluntary omission of exonic sequences in this study. The authors discuss that variants in the 5´- and 3´-UTRs were more interesting to study than coding variants as they think the levels of COX-2 might be more important than its activity. But this is a weak argument because any level of protein will likely be irrelevant without activity. Also, the authors themselves stressed the importance of extended haplotypes. Their postulate that these non-coding variants might be associated with COX-2 mRNA levels would be more convincing if these SNPs could be associated with any regulatory effect, for example on mRNA levels in lymphocytes (Stranger et al., Nat Genet 39:1217, 2007), or in regard of CpG islands, TF binding sites or DNAse hypersensitive sites.

Minor essential point: Provide a better rationale for the preferential analysis of
untranslated regions.

6. Are limitations of the work clearly stated?

It is now stated on page 16 that “The number of subjects in our case-control study was initially calculated considering the allele frequencies in the general population and a possible increase of 2 times this frequency among patients, with a significance level of 5% and an error level of 20%”. This is correct, though one could ask whether a two-fold increase would have been postulated a priori for any of the SNPs in COX-2. With other words, a negative result does not necessarily exclude the expected range of potentially low risks and therefore doesn’t tell us much. I understand that the authors have done their best to recruit as many patients and controls as possible. But the main argument for publishing slightly underpowered case-control studies of this kind (and there are many more around) would be the hope that they could prove useful in future meta-analyses.

Minor essential point: Discuss more critically the limitations of the present study size.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?

This has been improved. But the references are not always conclusive. It is discussed, for example, that “Vogel et al. [34] found no association between 8473TC polymorphism and breast cancer susceptibility, which was confirmed in three independent large studies [31, 39, 44]”. However, the latter three references appear to be on other cancers than breast cancer.

8. Do the title and abstract accurately convey what has been found?

This has been improved, since the discussion of age has been removed from the abstract. The title could be more precise if a term like “untranslated” or “regulatory” regions or something along these lines were included.

9. Is the writing acceptable?

It is acceptable. Nevertheless, the manuscript would benefit from a little language editing as some terms such as “comprehend” seem to be capable of being misunderstood and a few words seem to be misspelled (“infinity”, “cycles”).

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests