Reviewer's report

Title: A novel approach for rapid screening of mitochondrial D310 polymorphism

Version: 1 Date: 21 August 2005

Reviewer: Angela Tan

Reviewer's report:

General
Overall, there is a need to clarify what is the aim and hypotheses of the study. By revising the aim and hypotheses it maybe more understandable what final discussion and conclusions can be drawn from the results presented.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
None

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1. There are a few spelling mistakes and many grammatical errors in the manuscript, however, this does not significantly detract from understanding the study. The most notable are:
   - “breast cancer patients”
   - “16.6 kb” not “kbp”
   - “The D310…”
   - “gold standard”
   - The spacing of a number and the percentage sign should be consistent
   - There are other occasions where the use of “the” has not been used appropriately or not used when required
   It is advised to have the manuscript re-assessed for the use of more appropriate English terms for some phrases.

2. The question posed is not precisely clear from the abstract. The title is appropriate, but the application of this novel method is not immediately apparent. In the abstract, the Background section describes the D310 polymorphism in the context of a population study which is valid, but the Methods section describes cancer patients being screened by the novel technique. Perhaps it would be more relevant to describe the D310 polymorphism and its relevance to cancer progression or cancer screening in the Background section of the abstract.

3. The Methods section is not well described. The DNA extraction method does not appear to be complete and the reference to the DNA extraction method cannot be easily obtained. More detail after the centrifugation step is necessary. Please state the make and model of the PCR machine that was used. Indicating the manufacturer of the BsaXI or where it was purchased is also essential.

4. It would be clearer to the reader, if in the Methods section or the Results section, the recognition sequence of BsaXI was described. It is also not clear why the males were excluded from comparison with the breast cancer cases as mitochondrial DNA polymorphisms are maternally inherited. It is unclear what is meant by the statement “Therefore, it is not possible to determine the exact cytosine number”. Please clarify.
5. The Discussion section is not well developed. It is assumed that the aim was to develop a rapid method of detecting the D310 polymorphism as a tool to screen large numbers of cancer patients. Several points need to be addressed.

- There is no discussion of the possible reason why there was no significant difference between the tumour tissue and the matched normal tissues from each patient.
- In the patients where there was a difference between the tumour and matched normal, were these cancers a more developed cancer compared to the other samples?
- Is it possible that the observed transition from one BsaXI state to heteroplasmy in the tumour tissues be related to the selective amplification of another underlying mitochondrial population? (As it is described in the Sanches-Cespedes (2001) article).
- Is the finding of no heteroplasmy in the normal population expected? Could this be related to a detection sensitivity issue?
- Why would the BsaXI status relate to tumour development? In other words, why would there be a significant difference between the normal control BsaXI status and the colorectal tumour group?

Discretionary Revisions (which the author can choose to ignore)

1. If the BsaXI RFLP technique could be shown to be more sensitive at detecting the D310 7C carries than sequencing this would add more support for the technique. Perhaps an experiment could be performed using dilutions of known 7C carries with other C repeats and compared with the detection ability of sequencing.

2. Including the answers to the following questions is suggested.

- Why was a 19 hour digestion necessary? To ensure complete digestion?
- What sort of positive control for digestion is being used to prevent false negative results during the digestion step?
- Would the digestion profile of a 6C individual look like one from an 8C or 9C carrier?

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

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

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests