Reviewer's report

Title: A Case-Control Study of GST Polymorphisms and Arsenic Related Skin Lesions

Version: 1 Date: 10 July 2006

Reviewer: Alison Shield

Reviewer's report:

General

The authors have written an interesting paper reporting modest associations between several GST polymorphisms and arsenic related skin lesions. A number of studies have been undertaken in arsenic exposed populations at the association of various cancers with GST polymorphisms, including a recent similar paper investigating the GSTM1, GSTT1 and GSTP1 polymorphisms and skin-lesions (Ghosh et al. Int J Cancer 118: 2470-2478, published online 13 Dec 2005). Main differences between the current paper and the published articles include the size and location of the study population. Generally the study design and analyses are acceptable, however a few comments still need to be addressed prior to publication and are listed below.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. The referencing in the introduction is poor â€“ three sentences in the second paragraph are not referenced (beginning with â€œin vivo studiesâ€ and ending â€œformation of arsenic-GSH conjugatesâ€). Also reference 13 is stated to include animal data that shows GSH-conjugates being transported by MRP â€“ this reference does not show the stated data. The appropriate references should be included.

2. It is not entirely clear to the reader why the authors have chosen to study GSTT1, GSTM1 and GSTP1 polymorphisms. Arsenic biotransformation remains a complex mechanism, however it is generally accepted (since 2001) that GSTO1 is the rate-limiting enzyme for the GSH mediated reduction of arsenic. There is no evidence that GSTT1 or GSTM1 are involved in the conjugation of GSH with arsenic (or its transport). Although the authors cite the possibility of increased GST activity being associated with saturation of MRP and hence accumulation of arsenic the paper cited shows only an increase in GSTP1 levels with no change in GSTT1. The authors need to provide a clearer rationale for their choice of polymorphic candidates and why other polymorphic GSTs (such as the omega class) have been excluded.

3. In the methods section the authors have not clearly stated for the null polymorphisms how the genotypes were analysed. Have you modelled on allele frequencies, null homozygotes vs. pooled heterozygotes and WT, or pooled null homozygotes and heterozygotes vs. WT? Why havenâ€™t alternative models been discussed â€“ we might expect to see a dose response for 2 vs. 1 vs. 0 copies of the WT allele. Similarly, it is not clear from Table 1 whether we are looking at allele frequencies or genotypes (and why is this different for GSTP1).

4. Not being a statistician it is difficult for me to judge how robust the non-standard case-control design is â€“ this should be assessed by an expert statistician. It would also be useful to include a reference to a study using a similar approach rather than personal communications with one of the authors.

5. The sentence in paragraph 2 of the discussion is overstated â€œwe found that there was an increased risk of skin lesionsâ€. No evidence has been found for associations between GSTT1 and MRP (see also comment 2). More compelling evidence for GSTT1 needs to be given in the discussion â€“ particularly given an OR of only 1.56 and a null result in the competing Ghosh paper. Many of these enzymes belong to families with overlapping substrate specificities and are therefore it is likely that the null polymorphism is compensated for.

6. It would be useful for the authors to compare their findings with those of Ghosh et al; who did not find differences in GSTT1 and GSTP1 genotypes but did find a significant difference for GSTM1.
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. “Along™” has been superscripted for no apparent reason in the introduction.

2. You refer in the text to arsenic toenail concentration in Table 2. It is not clear in your methods how this was determined (or how it is applied in Table 2 analysis).

3. I could not find where you have defined the abbreviation LRTs.

4. It is confusing to talk about GSTs in the context of methylation “clarity about the role of GST in arsenic metabolism needs to be improved in the following discussion sentence: “while only GSTO1-1 has been implicated in arsenic methylation”

5. In the discussion “glutathione-θ-transferase” needs to be corrected to glutathione transferase θ.

Discretionary Revisions (which the author can choose to ignore)

1. Interestingly the active ingredient in betel nut inhibits various GSTs “it is possible that this may also be contributing to disease.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.