Author's response to reviews

Title: Ovarian cancer risk and common variation in the sex hormone-binding globulin gene: a population-based case-control study

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Author's response to reviews:

February 22, 2007

Dear Editor,

I would like to thank you and the reviewers for the careful review of our manuscript (Manuscript ID 4038082501213692). Below please find our response to the reviewers' comments. I have also uploaded a revised copy of the manuscript. In addition to changes suggested by the reviewers, we have added a co-author (Douglas Richesson) who was omitted by mistake from the original submission. Please, let me know if you have any further questions/comments about the manuscript.

Sincerely,

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Response to reviewer's comments

Reviewer's report
Ovarian cancer risk and common variation in the sex hormone-binding Title: globulin gene: a population-based case-control study
Version: 1 Date: 1 December 2006
Reviewer: Qiuyin Cai
Reviewer's report:
Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. Previous studies suggest that D356N polymorphism was associated with circulating levels of SHBG in post-menopausal women. Although the power is limited due to the small sample size, analyses stratified by menopausal status should be conducted.

We conducted these analyses and found no evidence for significant modification of the genotype associations by menopausal status (see attached Table at the end of the document). This has been added to the results section.

2. On the LD analysis between rs6559 and rs1799941 polymorphisms, the authors used both D’ and r2 methods. The results, however, contradict each other (D’=1 but r2 = 0.03). D’ analysis is not appropriate when frequency of a particular allele is very low.

We agree with the reviewer and have removed the D’ value.

3. In addition to haplotype analysis, it will be interesting to see if there is any joint effect of rs1799941 and rs6259 polymorphisms on the ovarian cancer risk.

Below are the requested analyses.

These analyses suggest that subjects with variant alleles in both SNPs are at particularly low risk; however the test for interaction was not statistically significant (P=0.149). We have added a sentence in the results section (paragraph 2) to summarize these analyses.

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. For the haplotype analysis (Table 3), the author did not use the highest frequent haplotype group (35% in control) as a reference group. Why?

We chose the haplotype with common alleles for each of the individual SNPs evaluated, rather than the most common haplotype as the reference category, to facilitate comparison with individual SNP analyses. We have added a footnote to the table to clarify the choice of the reference haplotype.

2. It will be helpful to present some background information for ATP1B2 gene. What is the function of this gene? Is there any functional interaction between SHBG and ATP1B2 genes?

The ATP1B2 gene was genotyped not because of its function, but because it was found to be in linkage disequilibrium with our gene of interest, SHBG, and we hypothesized that it could affect its regulation. However, to our knowledge there the functional interaction between SHBG and ATP1B2 has not been demonstrated. The enzyme encoded by ATP1B2 is involved in establishing and maintaining the electrochemical gradients of Na and K ions across the plasma membrane. This has been added to the introduction.

3. The title of Figure 1 states that the LD map is for the TP53 gene and its franking region. Is this a typo or did the authors use the wrong LD map?

This was a typo that has been corrected.
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. Background para 3 and Discussion line 1 - the authors claim that this is a 'comprehensive' evaluation. While I accept that the approach to SNP selection may have been comprehensive, I do not think that this claim is warranted for the study overall given the relatively small sample size.

The word "comprehensive" has been replaced by "detailed" to address this comment.

2. Methods para 1 - some additional information/clarification would help here. (i) Eligible cases were 437 women identified through hospitals and registries but it is unclear what proportion of ALL women diagnosed during the study period this represents. Would all women not identified through the participating hospitals have been identified through the registries? (ii) How complete are the population lists used to select controls? (iii) It would help to clarify that the numbers reported in the final sentence of this study were those that participated in the 'main' study (as opposed to the analyses reported here).

The 437 eligible cases represent all women diagnosed during the study period in Warsaw and Lodz, identified through the Cancer Registries. As the reviewer noted, all women not identified through our rapid ascertainment system at the participating hospitals, were identified through the Cancer Registries. The population lists are a complete census of the population. This has been clarified in the first paragraph of the Methods section. We also clarified that the 341 cases and 1994 controls at the end of the paragraph are participants in the main study.

3. Results para 1 - I do not understand the comment that 0% of clear cell cancers were poorly differentiated. Clear cell cancers are not routinely graded and, by convention, are usually all considered high grade. NB the proportion of clear cell cancers (22%) is also unusually high, most studies report 5-10% clear cell cancers.

As the reviewer pointed out, clear cell tumors were not graded, and therefore the 0% was an error, that has been corrected. There was also an error in the labels for histological types shown in Table 1. Where it said clear cell, it should have said endometrioid, and where it said mucinous it should have said clear cell. So, we have 14 (5%) of clear cell tumors in the study, which is within the range found in other studies. This error has been corrected.

4. Results Table 1 - given the role of SHBG it would be helpful to know what proportion of women had taken hormone replacement therapy. (NB. it would also have been interesting to evaluate the associations separately for hormone users and non-users - although I note that the number of oral contraceptive users was too small to look at this).

The distribution of HRT use has been added to Table 1. 35 (19%) cases and 70 (18%) of controls reported having ever used post-menopausal HRT. As the reviewer noted, the numbers are too small to evaluate genotype associations by HRT use.

5. Discussion paras 1 and/or 3 - as discussed above, I think some additional comment should be added regarding the lack of power to rule out even quite strong associations and, particularly, to look at interactions between genotype and environmental/behavioural factors.

We have extended our comment on limited power in the discussion (paragraph 3) to address this concern.

6. There are a number of typographical errors that should be corrected (eg is the rs number for the D356N SNP rs6259 or rs6559? Spelling of differentiated (not differenciated), untranslated (not untraslated)

Typos have been corrected.