Author's response to reviews

Title: Association of genetic polymorphisms in the interleukin-10 promoter with risk of prostate cancer in Chinese

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Author's response to reviews: see over
Dear Editors,

Please find attached a revision of our manuscript MS:2044090365374226, entitled “Association of genetic polymorphisms in the interleukin-10 promoter with risk of prostate cancer in Chinese”. Detailed below are the specific changes made in response to the reviewers’ and the editor’s comments. The authors greatly appreciate the reviewers’ careful readings of the manuscript and their constructive remarks. To directly address the reviewer’s comments, we have revised the Methodology, Results and Discussion sections. Specific attention has been placed on improving the description of the results, clarity of the tables, and grammar throughout the manuscript.

Point-by-point responses:

Reviewer 1:

1. rs no for all the three polymorphism should be mentioned.

We have added the rs no data for each of the three IL-10 polymorphisms into the Introduction. The rs no data are IL-10-1082(rs1800896), IL-10-819(rs1800871) and IL-10-592 (rs1800872).

2. In logistic regression analysis adjusted ORs for confounding factors like age, smoking etc. should be done.

The adjusted ORs for confounding factors like age and smoking status have been included in Table 3, and described in the Results section.

3. In the methodology section, version of SHE sis software’s version and country of origin should be given. Moreover, description of statistical approach used in the haplotyping software should be given briefly.

We have added the following description to the Methods section: SHEsis software is a powerful web-based platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci, which uses a Full-Precise-Iteration (FPI) algorithm to reconstruct haplotypes. The website http://analysis.bio-x.cn/ (Bio-X Inc.,Shanghai, China) is it’s Homepage.

4. What was average PSA in patients and controls? The authors have not specified the cut-off value for S.PSA level for the patients in their population, as they have also included patients having PSA levels less than 10ng/ml.
We have included information of PSA levels in the Results section. Average PSA levels in patients was 128.33 ± 634.21 (4.0-4165.0) ng /ml, compared to 2.146±0.954 (0.1~3.9)ng/ml in controls. PSA concentration of 4.0 ng/mL was considered a cut-off value to carry out a prostate cancer diagnosis.

5 The authors have made a major mistake in analyzing the results, 1st para, last 2 lines they have written that in their study they found higher percentage of patients with advanced stage than early stage cases (73% vs. 27%), whereas the table 1 showing demographic details reflect just opposite results with higher % of patients with early stage than advanced stage.

We thank the reviewer for identifying this error, and apologize for the mistake. The accurate data should have included 72 early stage cases and 180 advanced stage cases in our study. The percentage of advanced stage patients was higher than the percentage of early stage patients (68.7% vs. 27.5%). These corrections have been made in both the text and Tables 2,3 and 4.

6. In the materials and method section the authors define advanced prostate cancer cases as having either a Gleason score >7 or tumor-node-metastasis stage >T2. Hence they should also specify that did they calculated the no. of patients with aggressive status i.e. early or advanced because as written on combining the patients with gleason score >7 and TNM stage >T2 does not add upto the no. of 71 for advanced stage cases??????

Once again, we thank the reviewer for identifying our error. The accurate data is the following: 161(62.5%) cases were Gleason >7; 156(59.5%) cases were >T2 stage. We defined the “advanced prostate cancer” as either a Gleason score >7 or clinical T stage >T2. According to our defining, there were higher percentage of” advanced” (180 cases) than “early stage” (72 cases) in PCa patients (68.7% vs. 27.5%). These corrections have been made in both the text and Tables 2,3 and 4.

7. Next in table 4 which depicts the association of IL-10 haplotypes with Aggressive status the no. of patients with advanced stage has become 180 whereas that for the early stage is 72.......... Again changed from what is in the earlier results??

We have corrected this mistake as described in #6.
8. In discussion section a comparison of present results with other reports should be given which will provide a better understanding of the pathogenesis of the results.

We have revised the discussion section to include a comparison of other reports, including the following:
'Several prior studies have examined the relationship between PCa risk and genetic alterations in IL-10. The IL-10-1082 A allele, -819 TT and -592 AA genotype were associated with increased PCa risk (13-16), especially with high grade tumors (16). In contrast, no correlation was observed by Eder et al (17) and Michaud et al (18) at either the -592, -819, or -1082 SNP, or the promoter haplotype (ATA).'

9. As the results suggest the role of IL-10 -1082 in PCa metastasis and progression, it will be better if authors can provide a Kaplan Meier curve for time to HRPC associating it with IL-10 -1082 polymorphism.

We agree that Kaplan Meier survival would enhance our data set, however we regret that we do not yet have the survival data of these study subjects at present. Our results are preliminary, and further investigations such as the association of survival of HRPC and IL-10 polymorphisms will be done in the future.

10. The conclusions are inconclusive due to wrong projection of results in terms of tumor grade and metastasis state and mixing up of numbers between early and advanced stage tumor numbers.

We deeply apologize for the errors that were in the original submission. Thanks to careful review, we have eliminated all of these errors and have greatly enhanced the manuscript. We will take these mistakes to heart and use this experience to strengthen our writing skills. We hope that our revisions meet the reviewers standards, and that our manuscript be re-evaluates for publication. Thank you for your time and effort.

Reviewer 2:
This is a well conducted study that provides some interesting results. However, the value of the results could be increased by providing more context to the prostate cancer cases and how they might differ from those studied in Western countries in ways other than ethnicity. For example, many more of the men in this study had advanced than early stage prostate cancer. The opposite is usually observed in case-control studies conducted in areas where PSA
screening is commonly used. Additionally, despite repeated mention of the effects of the SNPs on IL-10 expression, no attempt was made to explain how SNP-induced changes in IL-10 expression might influence progression of prostate cancer as the authors suggest their findings indicate. Discussion of this point would strengthen the paper.

Major Compulsory Revisions

1. The controls are described as having no family history of cancer. Were they selected based on this factor? If so, this could introduce bias into the study because genetic factors and family history are related and individuals without a family history of cancer are likely to be at a different risk than those with a family history of cancer. If the controls were selected this way, the analysis should be redone with a new set of controls selected without consideration of their family history of cancer.

Controls were not selected by having no family history of cancer. As included into the Methods, Controls were screened to ensure that they had never been diagnosed with cancer or other serious disease and had low plasma prostate-specific antigen (PSA) levels (PSA<4.0 ng/ml), with the average PSA level 2.146±0.954 (0.1~3.9) ng/ml. The selected controls were matched to the cases by age (±5 years). Each selected subject was interviewed for family history of prostate cancer and smoking status. Analysis of family history of prostate cancer in case and control group revealed that there were more patients in the case group with a family history of PCa compared to controls, but the difference was not significant (5.3% vs 2.2%, P=0.067).

2. As described above, information on the methods by which the cases were diagnosed should be provided.

We have included a complete description of how diagnosis of the patient group was performed in the Method section. As described, the diagnosis of prostate cancer was based on digital rectal examination, serum Prostate specific antigen (PSA) concentration determination and transrectal ultrasound guided prostate biopsy. PSA concentration of 4.0 ng/mL was considered a cut-off value to carry out diagnostic work-up. Adenocarcinoma of the prostate was pathologically confirmed in all the cases, and the Gleason score (highly differentiated, score 2-5; moderately differentiated, score 6-7; poorly differentiated, score 8-10) was evaluated by pathologists working at each hospital using the Gleason scoring system (21). The clinical T stage of the patients with PCa was evaluated according to the 2002 TNM staging system for
cancer (22). We defined more aggressive and less aggressive disease based on tumor stage and Gleason score. “Advanced prostate cancer” was defined as either a Gleason score >7 or clinical T stage >T2.

3 Discussion of the possible mechanism(s) by which the SNP-induced change in IL-10 expression could influence prostate cancer progression should be added.

To discuss how SNP-induced changes in IL-10 expression might impact prostate cancer progression, we extensively revised the Discussion and included the following statements: While the precise mechanisms by which IL-10 polymorphisms may modulate PCa progression remains known, evidence suggests that IL-10 modulates immune function, such as NK cell, T cells, and macrophages activity, which would alter disease progression. Additionally, increasing evidence suggests a role for IL-10 in inhibition of angiogenesis, therefore decreases IL-10 expression would de-repress angiogenic activity and promote cancer progression.

4. Because the analyses were done using unconditional logistic regression, the matching factor (age) should be controlled for in the model. Other covariates, such as smoking, PSA screening, and diabetes status, should also be controlled for. Details of the covariates and what is included in the statistical analyses should be clearly stated.

We agree with the reviewer’s comments and have controlled for smoking status and PSA screening data for all study subjects in our analyses. Unfortunately, complete diabetes status data was not available and therefore we could not control for this variable. Changes made to the logistic regression analysis were described in the Methods, and data is presented in the Results.

Minor Essential Revisions

5. The authors should indicate whether the cases had any prior cancer diagnoses before their prostate cancer diagnosis.

All cases are the first confirmed prostate cancer diagnosis for each patient. This information is included into the Methods section under ‘Study subjects’.
6. A rationale for selection of the three SNPs that were studied should be provided.

Rationale for SNP selection has been added to the Introduction and Discussion sections.

7. In the second paragraph of the discussion, the TT at -819 and AA at -592 are referred to as alleles. These are genotypes. This mistake should be corrected.

Corrections have been made in the revised text.

8. Table 3 is referred to as Table 2 on page 4. This should also be corrected.

Corrections have been made in the revised text.

The authors greatly appreciate the editors and reviewers’ careful readings of our manuscript and constructive remarks. We hope that this extensively revised manuscript is suitable for publication.

Best regards,

Jie Liu