Author’s response to reviews

Title: Inflammatory Gene Variants and the Risk of Biliary Tract Cancers and Stones: A Population-Based Study in China

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Author’s response to reviews: see over
September 06, 2012

Manami Inoue, MD, Ph.D.
Editor BMC Cancer
Epidemiology and Prevention Division,
Research Center for Cancer Prevention and Screening,
National Cancer Center, Tokyo, Japan WC1E 6BT

Dear Dr. Manami Inoue,

Thank you for your interest in our manuscript, “Inflammatory Gene Variants and the Risk of Biliary Tract Cancers and Stones: A Population-Based Study in China.” The comments from the reviewers were quite helpful, and we believe the revisions have made this a stronger manuscript. Please find the revised manuscript and our point-by-point responses to the reviewer’s comments attached.

I look forward to hearing from you.

Sincerely,

Felipe A. Castro

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**Answer to Reviewers:**

**Ethical Approval:**

*We have included in the Methods section of the revised manuscript an statement for the Ethical Approval. (page 5).*

**Reviewer:** Dipti Chourasia

**Reviewer's report:**

The article is precisely written and presents a few novel findings and hence adds knowledge to the present information available. However, there are some queries, which need to be addressed:

1. Abstract: Fine
2. Introduction: Sufficient
3. Material and Methods:
   
   Study participants: Authors mentioned the year of inclusion of patients for this study as 1997-2000. However, in their previous study reported (Hsing AW et al.,2008, Cancer Research), they mentioned the same year interval for inclusion of patients. They reported the total no. of patients with biliary tract cancer as 411, whereas in this study it is 456. This indicates a selection bias. Further, in their previous study, authors reported 1037 biliary stone cases. Whereas they mentioned just 982 such cases in this study. Is this sample variation due to lack of sufficient sample left for this study and hence authors had to remove those cases. If this is the case, authors have to mention this clearly in this material and methods section (Discretionary Revision).

4. Results: Precise
5. Discussion: To the point

**Our Answer:**

*Differences in the number of subjects included in this and previous studies were due to availability of DNA samples and genotyping completion rates since genotyping for this study was completed subsequent to our previous study. Therefore, selection bias should not be a concern; the distribution of demographic characteristics in this study and our previous study is essentially the same. We have included an additional explanation in the statistical analysis section of Material and Methods (page 6, second paragraph): “The final analysis included subjects who completed the interview and for whom we had DNA samples and genotyping results. A total of 456 biliary tract cancer cases (262 gallbladder, 141 extrahepatic bile duct and 53 ampulla of Vater), 982 biliary stone cases (252 bile duct, 730 gallstone), and 860 controls, were included.”*
Reviewer: Kshitij Srivastava

Reviewer's report:

Minor Essential Revisions

In the present study, Castro et al. investigated the putative association of inflammatory gene polymorphisms with biliary stones and biliary tract cancer risk. A total of 35 polymorphisms in 5 genes were evaluated. The study provides further support to previous reports for the importance of inflammatory pathway in cholelithiasis and biliary tract carcinogenesis. The authors found significant associations with SNPs in VEGFA with biliary stones, IL8 with gallbladder and ampulla of Vater cancers, and RNASEL with ampulla of Vater cancer. Although larger studies are still needed to provide a more comprehensive view, the major weakness of the present study is the lack of information on the functional effect of the associated SNPs. Although the authors have stated for IL8 rs10805066 SNP that no functional information has been reported, the functional effects for significant SNPs in RNASEL and VEGFA have not been mentioned/discussed. It will be useful to add the relevant information.

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Our Answer:

We have meticulously searched for any predicted functionality of these variants but have not found evidence of gene or transcript consequences (biological effects in transcription). We have added a few words to clarify this point in the discussion section (page 10, first paragraph).
Reviewer: Balraj Mittal

Reviewer's report:

Major Compulsory revision: None

Minor essential revision:

The study design, results and conclusions are fine. However, the Discussion appears a bit mechanical. It can focus more in-depth role of inflammation in biliary diseases, The authors did not get any differential association in patients with/without gallstones but inflammatory pathway is mainly linked to stone-induced cancer. Therefore, these points should be discussed. Also, the inflammatory cascade from already known or new genes from this study may be proposed.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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

Our Answer:

We added a paragraph in the Discussion on page 9 addressing the point that the associations were non-differential in patients with or without stones. We also added suggested inflammatory-related events in biliary disease etiology, but the cascade of events in relation to the genes detected in this or previous studies, with or without the presence of biliary stones, is unclear.