Author's response to reviews

Title: Association of an NFKB1 intron SNP (rs4648068) with gastric cancer patients in Chinese Han population

Authors:

renquan lu (lurenquan66@hotmail.com)
Xiang gao (gxcy2001@hotmail.com)
Yin Chen (1251487447@qq.com)
Ran Xiao (ljerry19890505@hotmail.com)
Jian Ni (jiannihome@yahoo.com)
Sheng Li (lisheng1996@gmail.com)
Lin Guo (jykca@sina.com)

Version: 3 Date: 27 February 2012

Author's response to reviews: see over
Dear Mr Mark Cardinez,

We are pleased to receive your letter and thank for the reviewers’ work for our manuscript. To address reviewers’ concern, we carried out the related revision and the point-to-point answer is as follows:

**Answers for Review 1 (2322974316528489_comment):**

**Major Compulsory Revisions:**

Patient characteristics of the cancer vs control patients are not shown. Please include a table of age and sex demographics, and an evaluation of balance between groups.

Answer: Thank you for your suggestion. We’ve added the information of the cohort, including age and gender demographics, and the comparison.

**Minor Essential Revisions:**

1. Throughout the manuscript (including the title) the authors refer to their gene of interest using the protein name, NF-KB 1. All references to the gene should be as NFKB1 (italicised). The manuscript contains editing errors related to; spacing, gene names not shown in italics and inconsistent presentation of protein names.

Answer: Thank you for your correction. We have revised them accordingly.

2. Background section, paragraph 3. Please remove the results of the pilot genotyping study from this section, since this is repetitive of what is covered in the results section.

Answer: Thank you for your correction. The pilot genotyping study has been removed from Background section, paragraph 3.

3. Methods section, paragraph 2. MJ Research have “Opticon” thermal cyclers, not “Option”. Please check correct name of instrumentation.

Answer: Thank you for your correction. This “Option” error has been corrected.

4. Methods section, paragraph 3. The statistical analysis description lacks sufficient detail to determine which measures were applied to each analysis.

Answer: Thank you for your correction. We have revised this paragraph about the statistic analysis.

5. Results section, paragraph 2. Change extron to exon Results section, paragraph 4. Change from (Table 4) to (Table 3).

Answer: Thank you for your correction. The errors have been corrected.

6. Discretionary Revisions: Include 3 – 10 keywords

Answer: Thank you for your suggestion. We have added some keywords: NFKB1, gastric cancer, single nucleotide polymorphism (SNP), susceptibility.

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

**Quality of written English:** Not suitable for publication unless extensively edited.

Answer: Sorry for the trouble we are bringing. We have done our best to revise the manuscript. Therefore, we’d liked to asking for Edanz service to correct our language if our revision and answers were thought to be suitable.

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

**Answers for Review 2 (3194540286635669_comment):**

**Version:** 2 **Date:** 16 January 2012

**Reviewer:** Megan Hitchins

**Reviewer's report:**

Lu and colleagues have performed a straightforward case-control study exploring the SNP genotype and allele frequencies in a gastric cancer cohort and appropriately matched healthy control population of Chinese Han
individuals to test for associations with particular SNP alleles within the NF-kB1 gene and gastric cancer. This study, although simple in concept, design and execution, has yielded an exciting finding worthy of reporting. Lu et al. demonstrate an association between gastric cancer incidence, as well as particular clinico-pathologic features among gastric cancers, and an intronic SNP genotype within NF-kB1. They tested 4 SNPs in a subset of their individuals (30 cases and 30 controls) and demonstrate that the homozygous genotype for the minor allele (among healthy controls) of intronic SNP rs4648086 is associated with an increased risk for gastric cancer. They then extended their study for this SNP alone to the rest of their cases and controls. This seems logical and cost-effective. However, it is interesting to note that even among the initial test case-control group studied, another SNP (rs4648065) showed a trend but did not reach statistical significance. It is plausible that with inclusion of additional individuals, and hence an increase in the power this would have afforded, this SNP may also have reached significance. Since these 2 SNPs have distinct SNP-tags (ie may or may not be in particularly close linkage disequilibrium) the authors may have missed some important information.

Therefore, the paper should provide (minor essential revisions):

1. A better justification for selecting only the one SNP for extended study ie provide projected power calculations for the extended cohort on the basis of the initial test study to warrant the strategy they employed in only studying one SNP further.

Answer: Thank you for suggestion. It is very instructive. We found that among 4 SNPs, only rs4648068 exhibited a suitable P value. That is why we chose it in the further study. Maybe we were a little strict towards rs4648065. We agree that rs4648065 might reach significance, and should be studied. Now, we can only take this SNP into consideration in the future study. To address this issue, we have to address it with the following sentence in the discussion: “we found that rs4648068 polymorphism showed significant difference of the distribution between gastric cancer patients as compared with the health control (P = 0.037)”.

2. Provide an assessment of the haplotypes they identified among the 4 closely located SNPs in the 30 cases and 30 controls. This should include the possible combinations (43 = 12), and which ones they are likely to have observed on a probabilistic level by calculating R2 values among their observed genotypes. This would certainly be possible by studying homozygotes. Otherwise, programs such as PHASE could be used for this. Then, if they re-calculate their associations based on these likely haplotype frequencies as well, they may explain why each of the SNPs showed a trend towards significance. It is likely that the lower the P value among the other 3 SNPs, the closer it is located to the intronic SNP rs4648086. There is always a danger with SNP association studies that any one SNP is tagging another site. By studying the haplotypes in total, this would provide greater evidence that the rs4648086 SNP may indeed be causally related to the phenotype (not just linked to something close by that is functional). A recent study on VHL in clear cell renal carcinoma provides a good model for the extra statistical work this would entail. This study is worthy of publication if the issues above are adequately addressed. This would require no further lab work, but additional data mining.

Answer: Thank you for your suggestion. We would be so pleased if we could hear from you before. We have tried downloading PHASE based on your suggestion, but unfortunately failed (user name and password were required to do so). We are going to invite some good statisticians to join us in the next study. You are heartily welcome as an advisor for our research group. The goal of our study is to bring attention to NF-κB and its SNPs. And we sincerely hope that more scientific research would be spent on them by those who are more professional in statistics. We would like to cooperate with those who are interested in the SNP and other tumor study on Chinese Han population.

The authors should also take into consideration some minor points for inclusion in their paper, which should improve the overall content (discretionary revisions):

1. A scaled map (as supplementary information if not in the actual text) should be drawn to provide an overview of
the SNP locations with respect to their position within the gene, with respect to one-another, and if possible, also their tag-SNPs.

Answer: Thank you for your correction. A diagram to show genetic association between 4 SNPs of NFKB1 genomic region in the Chinese Han population has been added into revised manuscript, with necessary description.

2. Odd Ratios and CIs should be provided in all the Tables, where relevant, not just the frequencies and P values. One OR is referred to in the Discussion, but is no-where in the Results. The Adjusted odds ratios could be provided as ‘AOR’.

Answer: Thank you for your suggestion. We have revised the table to comply the recommendation.

3. A sentence or two should be added to the Discussion to explain why, although the P values are significant, the ORs are low, to provide an interpretation of the degree of contribution.

Answer: Thank you for your suggestion. We have added some sentences: the SNP within NFKB1 intron region (rs4648068) showed significant but weaker ORs, these data suggest that there might be some potential correlation between NFKB1 genotypes and the certain gastric cancer clinic-pathologic characters such as lymph node status and serosa invasion, due to the ORs significantly differ between the high- and low-risk subsets.

4. The potential functional role of the intronic SNP could be speculated upon further so the paper is hypothesis-forming as well as merely reporting a simple finding. For instance, some interrogation of the databases might reveal something worthy of further investigation: cross-species conservation analysis of the non-coding region where the key SNP is located might reveal it is located in a conserved region, which would provide supportive evidence for a functional role eg within an enhancer element for example. The Encode database could also be examined at this location to determine if this SNP might be located within a regulatory element eg is it associated with increased levels of H3K9me1 (monomethylation) that is typically located at enhancer elements.

Answer: Thank you for your suggestion. These are great ideas. We’ll try and put these suggestions in our future study.

Level of interest: An article of importance in its field.

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

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

Declaration of competing interests: I have no competing interests to declare

Answers for Review 3 (1773184919665650_comment):

Reviewer's report

Version: 2 Date: 20 January 2012

Reviewer: Jyotsna Batra

Reviewer's report:

The current study has been conducted on a very small no of samples, so doesn't have adequate power to reach to a conclusion, thus is unacceptable unless is replicated in another cohort. please see my response to the editorial Q's below:

1. Is the question posed by the authors well defined? Yes

2. Are the methods appropriate and well described? No, small sample size and inadequate power.

3. Are the data sound? NO, lack of power.

Answer: Thank you for your suggestions. We agree that the study would be more convincing if it were replicated in another cohort. We will try to find another source of patients for this study in further critically examine our finding in another related project/grant. Here, in this revision we have acknowledged the limitation of our study by adding a sentence: “Although the MAF of rs4648068 is high (0.346) that had given some evidences for conclusion,
we would like to point out a limitation of our study that the conclusion would be more convincing if it were replicated in another cohort.”

4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes.
5. Are the discussion and conclusions well balanced and adequately supported by the data? Yes.
6. Are limitations of the work clearly stated? Partly.
7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes.
8. Do the title and abstract accurately convey what has been found? Yes, but the conclusion is not imperative due to lack of the power.
9. Is the writing acceptable? Yes.

Additionally, we also do some revisions to fit for the format of the journal. We have done our best to revise the manuscript. We are willing to ask for Edanz service to correct our language if our revision and answers were thought to be suitable.

We sincerely hope that these answers could address the reviewer’s concern.

Yours sincerely,

Lin Guo (Prof.) and Renquan Lu (Dr.)