Reviewers comments

Reviewer: 1

Q1. The authors could have examined the gastric specimens in greater detail, i.e., graded the degree of gastritis, intestinal metaplasia, etc . using the Sydney system (-, +, ++, +++). Instead, the data is presented as lesion present or not present (yes or no).

We revised it as the reviewer suggested (Table 2)

Q2. Also, since blood samples are available, why not measure the levels of IL-21 in the blood? The presence of antibodies against H. pylori and the CagA toxin could also be measured in the blood and related to IL-21 SNPs.

This is a perfectly reasonable suggestion. To our knowledge, ours is the first study of this issue. Therefore, given the unknown nature of the association, as well as our modest sample size of 878 participants, our primary aim was to establish whether there was, in fact, any association with gastric precancerous lesions. Now that we have established a possible causal association with certain genotypes. For reasons stated above, Q2 was not our primary aim, which was to address the most fundamental hypothesis – is there an association between specific genotypes and the presence or absence of gastric precancerous lesions. Also, as mentioned above, analyses that increase the number of potential effect-modifying categories will benefit from a larger sample size. Therefore, we will conduct these analyses in future studies.

Q3. The study is fine and publishable in its present form, but this reviewer feels that more could have been done with the samples available to the investigators.

Thank you. Please see replies above.

Reviewer: 2

Q1. -How do you calculate power and sample size calculation? -The number of controls subject must be a least equal to patient?

In this study, 290 controls and patients with NAG (116), AG (306) and IM (366) were selected according to the inclusion and exclusion criteria. In the study design stage, the ratio of cases and controls we initially designed was 1:1, but only 116 NAG cases met the inclusion criteria. Finally, we compared the control group with the three case groups, and compared the control group with the whole case group.

Q2.-You must add new control subjects and you must do new genotyping when you made a comparison between controls and intestinal metaplasia groups - New association must be indicated and discussed;

We are sorry about that it is difficult to add a new control group at present, because the recruitment stage of this project has been completed. However, we have determined the inclusion criteria of the control group in the study design, and we believe that the difference between the controls and cases can be tested according to the current study design.

Q3.- Haplotype analysis must be done.

Thank you for your suggestion, we have added it (Table 5; RESULTS—last paragraph; DISCUSSION—fourth paragraph).

Q4.- what is the advantage to use the polymerase chain reaction–ligase detection reaction (PCR-LDR) method"

The PCR-LDR DNA genotyping technique is simple, highly accurate, has high-throughput, and is cost-effective[1]. The PCR-LDR method can also reduce false-positives and eliminate the need for both post-PCR and post-ligation purifications，and could provide high specificity and sensitivity in

SNP analyses.

[1] Luo Y, Tang S, Gao W, et al. (2010) Genotyping mitochondrial DNA single nucleotide polymorphisms by PCR ligase detection reactions. Clin Chem Lab Med 48(4): 475-483.

Q5.- Exact sequences of oligonuleotide primers and probes must be added with references .

Thank you for your suggestion, we have added it (Table 1; METHODS—Genotyping: Line 6-10).

Q6.- Condition to conduct sequencing analysis must be added and the sequencing profil for each genotype of each SNP must be added as a new figures

According to the aim of this study, we have carried out SNP genotyping, so as to detect SNP site information, we have not carried out gene sequencing analysis.

Q7.-Please reformulate this sentences " Analyses of rs907715 mRNA expression in intestinal epithelium tissue from six subjects with IM showed a monotonic inverse association with copies of the C allele ($p<0.001$)".

Thank you for your suggestion, we have reformulated it. –"Analyses of rs907715 mRNA expression in intestinal epithelium tissue from six subjects with IM showed a significant difference between CC genotype and TT genotype ($p<0.01$), CC genotype and CT genotype ($p<0.01$)."

Q8.- How do you perform the mRNA expression study? please indicate all steps from extraction to quantification with details in manuscripts.

Thank you for your suggestion, we have added it (METHODS—Genotyping: last paragraph).

Q9. -In order to better understand the role of IL-22, the IL-22 mRNA expression must be done between cases and controls. - The numbers of IL-22 mRNA expression samples must important and equal for each gastric precancerous lesions groups -The IL-22 mRNA expression must be studied according to different genotypes for each polymorphisms and between case and control groups. - New results based in the correlation between IL-22 mRNA expression and Il 22 SNPs must indicated and discussed.

Thank you for your suggestion. The aim of this study is to explore the difference between three SNPs and gastric precancerous lesions. After analyzing our data and finding the distribution difference of rs907715 genotypes (CC, CT, TT) in IM patients, we explored the difference of mRNA expression of rs907715 genotypes in six IM tissues. The results showed that there were significant differences in mRNA expression between CC and CT, CC and TT of rs907715 ($p<0.01$), which further confirmed our findings (the C genotype of rs907715 is related to the occurrence of IM)

Since the other two SNPs (rs12508721, rs2221903) were not found to be related to gastric precancerous lesions in this study, the difference of mRNA expression in different genotypes for the two SNPs was not further explored. However, we have previously studied mRNA expression of rs907715 in AG and NAG patients, which was not shown in the previous manuscript because our main focus is on the relationship between rs907715 and IM patients, this part of the data has now been added based on your suggestions(Figure3 and Figure 4).

Q10.- statistical methods and test used to study the IL-22 mRNA expression must indicated

Thank you for your suggestion, we have added it (Statistical analysis: Line 8-11).

Q11.- The title of fig 2 "-21 mRNA expression level in 6 intestinal epithelium

tissues as function of rs907715 genotypes."must be corrected.

Thank you for your suggestion, we have revised it—"IL-21 mRNA expression level in 6 intestinal epithelium tissues among three rs907715 genotypes".

Q12.-References must be updated.

Thank you for your suggestion, we have updated it.