November 4, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5329-edited-sun Revised.doc).

Title: Genetic polymorphisms of miRNA and risk of gastric cancer in Asian population

Author: Haibing Hua, Tingting Yan, Qingmin Sun

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 5329

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

REVIEWER 02454001:

The article is good, but as this is a review article it can be more exhaustive. please include these references- as the Biogenesis was described by Kaiser Jamil Including an appropriate diagram. .Kaiser Jamil (2007) “MiRNAs Rewrite the Rules of Molecular Biology” -Research Journal of BioTechnology Vol.-2 (4), 3 – 5. 2. Gowhar Shafi, Kaiser Jamil, Atya Kapley and Hemant Purohit.(2009) RNAi as a Novel Therapeutic Platform Technology for Oncological Solutions- a review. Biotechnology and Molecular Biology Reviews Vol. 4 (3), pp. 055–070, June 2009.

Response:

We appreciate the reviewer’s encouraging comments. As suggested, we have included and cited these references in our manuscript. It will help us to recognize the miRNAs biogenesis more clearly.

Reviewed 02458583:

The manuscript looks unaligned and hard to focus in some sections. Search strategy and the method of analysis are not clear. Systematic approach is needed to have clear conclusions.

Response:

Thanks for your suggestion. As suggested, we added the search strategy and the method of analysis as follows:

“In our present study, we focused on the miRNA SNPs in gastric cancer susceptibility.

Therefore, we searched PubMed through August 2013 to identify all relevant papers, using the key words microRNA, miRNA polymorphism or variant in combination with gastric cancer or gastric tumor. There were about 35 epidemiologic studies were carried out that focus on the importance of miRNA-related SNPs in gastric cancer susceptibility. Then, we scanned the titles and abstracts and excluded the studies that were clearly irrelevant to the current topic. The remaining articles were read to determine whether they contained information of interest. In addition, to be eligible, studies had to fulfill the following criteria: 1) a case-control study design or prospective study design; 2) reported the number of subjects with each allele or genotype in cases and controls; 3) reported the risk estimates [odds ratio (OR)] and 95% confidence intervals (CI) or provided raw data that allowed us to calculate them; 4) the risk estimate was only with miRNA polymorphism, not with miRNA binding site SNPs. All searches were performed independently by two investigators. As result, 13 epidemiologic studies were included in our study."

"Additionally, to ensure the pooled-analysis is more accurate, we separated the numbers of subjects with each genotype in all included studies. Subsequently, the numbers of each genotype were summed as an overall study, and directly calculated the OR and 95% CI based on the most frequently adopted genetic model in original literatures."

These sentences also were added in our manuscript.

Reviewed by 02494487

This is a review of published literature on the effect of miRNA on the risk of gastric cancer. Further a polled analyses of 3 polymorphisms is attempted. The manuscript is inappropraitely titled as it is specific for Asian populations only (table 1). There is no methodology indiacted for pooled analysis, is it meta-analyses? If so, what criteria was used for selection of studies? English editing is strongly recommended and authors should also keep terminology consistent eg. MiRNA vs MIRNA

Response:

Thanks for your valuable suggestion. In fact, in our search strategy, we don't limit the studies only in the Asian population. We searched all published English literatures in the database of Pubmed, and the published results specially focused on the Asian population. It maybe that gastric cancer remains the highest rates of incidence and mortality in Asian, which lead to more researches about gastric cancer. However, to be more accurately, we also revised the title as "Genetic polymorphisms of miRNA and risk of gastric cancer in Asian population".

In the present study, our pooled analysis is similar to the meta-analyses, but to ensure the conclusion is more valuable, we directly calculated the OR and 95% CI based on overall numbers of each genotype instead of pooled OR. And the search strategy and criteria as well as method of analysis, we have added them in the fulltext, as follows:

"In our present study, we focused on the miRNA SNPs in gastric cancer susceptibility. Therefore, we searched PubMed through August 2013 to identify all relevant papers, using the key words microRNA, miRNA polymorphism or variant in combination with gastric cancer or gastric tumor. There were about 35 epidemiologic studies were carried out that focus on the importance of miRNA-related SNPs in gastric cancer susceptibility. Then, we scanned the titles and abstracts and excluded the studies that were clearly irrelevant to the current topic. The remaining articles were read to determine whether they contained information of interest. In addition, to be eligible, studies had to fulfill the following criteria: 1) a case-control study design or prospective study design; 2) reported the number of subjects with each allele or genotype in cases and controls; 3) reported the risk estimates [odds ratio (OR)] and 95% confidence intervals (CI) or provided raw data that allowed us to calculate them; 4) the risk estimate was only with miRNA polymorphism, not with miRNA binding site SNPs. All searches were performed independently by two investigators. As result, 13 epidemiologic studies were included in our study."

"Additionally, to ensure the pooled-analysis is more accurate, we separated the numbers of subjects with each genotype in all included studies. Subsequently, the numbers of each genotype were summed as an overall study, and directly calculated the OR and 95% CI based on the most frequently adopted genetic model in original literatures."

In addition, we have revised the MIRNA as MiRNA.

Special thanks to these good comments.

Reviewed by 00011378

The MS of Hua et al as a topic highlights about SNPs of miRNAs and the risk of gastric cancer is timely and on a hot topic of research. Despite that authors have addressed some comments of other reviewers, major concerns still arise. In the abstract and introduction, authors mentioned the common view that miRNAs regulates negatively gene expression though the opposite has been described and should mentioned. The language needs revision preferably by a native English-speaker or Professional editing service, i.e. some verbs are in present time talking about reported work, which should be in simple past, second paragraph of the introduction, "identify should be identified".

Response:

Thanks for your valuable suggestion. As suggested, the language has been revised by the

professional editing service.

Other examples throughout the MS are "subject with variant homozygote CC" that should be replaced by subject homozygous for the variant C" and other versions of that.

Response: We have revised it, thanks.

From some pharses the reader may have wrong information, i.e.in the section MIRNA BIOSYNTHESIS AND FUNCTION, authors affirm that pri-miRNA are 400 bp long which is not always true.

Response:

Thanks for your suggestion. In our manuscript, we referred the sentence from *Cancer Lett. 2011; 300(1): 10–19*, and the referred sentence is "An intron of about 400 nucleotides is excised from the primary transcript and becomes the primary miRNA (pri-miRNA)". According to your kindly advice, we searched literatures again, and found that most studies didn't indicate the accurate length about pri-miRNA. Therefore, we revised it in the fulltext, many thanks.

But the major concern arise about the pooling methodology for including the studies referred to the 3 main SNPs discussed. The methodology used by the authors has many caveats. Then, The analysis should be redone using classical meta-analysis approaches using Mantel Haenszel ORs or another methods to weight each studies and to obtained the pooled effect assuming i.e. fixed effects. Other approach, which can be done simultaneously, is using the estimation of random effects. In doing so, it is possible to identify heterogeneity, publication bias and another factors associated with the analysis. Saying that the results described as the main conclusion of the MS are weak. Of course, it is not acceptable say that the results showed a tendency when the results are not significant, i.e. for SNP rs11614913 in miR-196a-2.

Response:

Thanks for your suggestion. As suggested, we redid the results using classical meta-analysis approaches. As follows,

The Z-test was used to determine the statistical significance of the pooled OR, and $P < 0.05$ was considered as statistically significant. Additionally, Q and I^2 statistic were used to examine possible heterogeneity of study results. [1] If significant heterogeneity existed ($P < 0.10$ was considered representative of statistically significant heterogeneity), the pooled OR estimate of each study was calculated by the random effects model, otherwise the fixed-effects model was used.

The revised results were also updated in manuscript.

In addition, only 3 to 5 potentially relevant studies were included in each meta-analysis study; therefore, it's difficult to further define the heterogeneity and publication bias.

In addition, authors confused OR with Risk, which have different forms of calculation and are not exactly the same i.e. OR=1.58 does not means a 58% increased risk.

Response: Thanks for your suggestion, we have revised it.

For the SNP rs895819 in miR-27a a minor allele C is mentioned opposed to the A/G variant described earlier (this reviewer assumes that C corresponds to G in the opposite strand but this should be clarified).

Response: A-G means A and G are the polymorphic bases on one strand. These are equivalent to T and C on the other strand. (ftp://ftp.ncbi.nih.gov/snp/database/Illumina_top_bot_strand.note.txt). As suggested, we also clarified it on the manuscript.

In the description of the study of Ahn et al (49) about miR-146a, the description of genotypes is confusing or directly wrong.

Response: Thanks very much for your careful correction; I am sorry for this mistake because of the lapsus calami in original table 2 by Ahn et al. We have revised it, thanks.

Reviewed by 00207727

Response:

Thanks for your good suggestion; in fact, we have conducted many meta-analysis studies in recent years [1-4]. Given the small-scale studies were included in our study; we performed a simple pool analysis. However, as suggested, we redid the results using classical meta-analysis approaches, and carefully re-calculated the conclusion based on the currently available data, many thanks.

Reference:

1. Sun Q, Xu L, Zhou B, et al. Alcohol consumption and the risk of endometrial cancer: a meta-analysis. Asia Pac J Clin Nutr. 2011;20(1):125-33.

2. Jing YL, Sun QM, Bi Y, et al. SLC30A8 polymorphism and type 2 diabetes risk: evidence from 27 study groups. Nutr Metab Cardiovasc Dis. 2011 Jun;21(6):398-405.

3. Zhou B, Sun Q, Cong R, et al. Hormone replacement therapy and ovarian cancer risk: a meta-analysis. Gynecol Oncol. 2008 Mar;108(3):641-51.

4. Zhou B, Yang L, Sun Q, et al. Cigarette smoking and the risk of endometrial cancer: a meta-analysis. Am J Med. 2008 Jun;121(6):501-508.e3.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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