Author's response to reviews

Title: Common Variants in Mismatch Repair Genes Associated with Increased Risk of Sperm DNA Damage and Male Infertility

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

Thank you for your letter on 13-MAY-2012, in which you encouraged us to make some revisions to our manuscript (MS: 1175193129623197), entitled "Common Variants in Mismatch Repair Genes Associated with Increased Risk of Sperm DNA Damage and Male Infertility ".

Many thanks for the editor’s comments and suggestions. The suggestions are quite helpful for us. We have modified the manuscript accordingly, and the detailed corrections are listed below point by point.

We deeply hope our responses are satisfactory. If you have further questions, please let us know by E-mail. Thank you again for your consideration and we await a favorable response to the revision.

Thanks and Best Regards!

Yours Sincerely,

Guixiang Ji
2012/3/19

Responses to the quests:

>1. Data should undergo a Bonferroni correction (ref 1)- we are satisfied with your response to this point in your covering letter, but you need to include this information in the manuscript itself.
   Thanks for the editor’s suggestion. We have added this information in the “Statistical analyses” section on page 11. Please find the changes in the revision.

>2. Actual SNP rates are low; this needs to be better discussed (ref 1). Again, you have mentioned this in your covering letter, but it is not discussed or better emphasised in the manuscript- please do this.
   Thanks for the editor’s advice. “Although, these three SNP (rs4647269, rs1059060 and rs2075789) associated with risk in our study are significantly higher for some variants in the patient group, the actual rates are quite low and would potentially account for a low percentage of infertility. However, it is important to know that genetic variants associated with common complex diseases like male infertility are only “one piece of the puzzle” making up an individual’s overall risk for disease. It is highly likely that the genetic risk for developing male infertility is influenced by the additive effects of many different genetic variants and other risk factors. So, further
research is required to define their interactions with other susceptibility alleles and environmental factors can lead to a substantial increase in male infertility risk, especially when exposed to certain dietary and lifestyle habits.” This information has been added in “Conclusions” section in page 18-19.

>3. Provide information about cryopreservation and discuss whether it affected sperm (ref 1)- this also needs to be included in the manuscript itself.

Many thanks for the editor’s wonderful suggestion. “In the present study, semen samples were pre-treated with cryopreservation prior to TUNEL analyses. However, it has been demonstrated that the process of cryopreservation can led to an increase in oxidative stress and percentage DNA fragmentation [27].” This information has been added in “Results” section in page 13.

>4. It is well known that cryopreservation resulted in a significant increase in percentage sperm DNA fragmentation (Thomson et al. Hum Reprod 2009;24: 2061-2070). Did the authors evaluate the cryopreservation effect on DNA fragmentation in this experiment?- again, please include the table and discussion from your covering letter in the manuscript itself.

Thanks for this comments. “To determine whether the results of the TUNEL analyses were profoundly influenced by cryopreservation in our study, ten semen samples were pre-treated with or without cryopreservation prior to TUNEL analyses. As showed in Additional file 3 (Table S3), modest but significant elevated levels of sperm DNA fragmentation induced by cryopreservation \( (P = 0.001) \). However, all the semen samples undergo the same cryopreservation process, thus we believe that the cryopreservation, if any, is unlikely to be substantial.” This information has been added in “Results” section in page 13.

>5. Authors' contributions (editorial request): you have confirmed in your covering letter that YL and CH were involved in the drafting of the manuscript, but this information must be included in the manuscript itself (in the Authors' contributions section)

Thanks for the editor’s correction. We have clarified that YL and CH were involved in the drafting of the manuscript. Please find them in the revision.

>6. STROBE guidelines: your covering letter states that your manuscript now adheres to the STROBE reporting guidelines, but parts are still missing. The checklists can be found here: [http://www.strobe-statement.org/index.php?id=available-checklists](http://www.strobe-statement.org/index.php?id=available-checklists)

> Please ensure that ALL points are included in your manuscript; for example, the study type should be identified in the title or abstract, and sources of bias/limitations should be addressed. Please go down the list and ensure all STROBE items are addressed.

Thanks for the editor’s remind. “We selected and genotyped 21 tagging single nucleotide polymorphisms (SNPs) in five MMR genes (MLH1, MLH3, PMS2, MSH4 and MSH5) using the SNPstream 12-plex platform in a case-control study of 1,292
idiopathic infertility patients and 480 fertile controls in a Chinese population.” This information has been added in “Abstract” section in page 2.

“Of note, like all case-control studies, selection bias may exist and might influence interpretation of the results. However, we believe that potential confounding bias might have been minimized by matching the controls to the cases on age and by further adjustment for the confounding factors in statistical analyses. In addition, the fact that genotype frequencies of all SNPs in our controls fit Hardy-Weinberg equilibrium and were similar to those obtained from the HapMap Project further supports the randomness of our control selection. We believe that the selection bias, if any, is unlikely to be substantial.” This information has been added in the last paragraph of “Discussion” section in page 18.

We deeply hope our responses are satisfactory. If you have further questions, please let us know by E-mail. Thank you again for your consideration and we await a favorable response to the revision.