Final Decision made for SREP-16-10787-T

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附　件 :

Dear Dr Guo:

Thank you for your help with manuscript SREP-16-10787-T, "Tertiary Epimutations - A Novel Aspect of Epigenetic Transgenerational Inheritance Promoting Genome Instability", which you recently reviewed for Scientific Reports.

For your records, the decision for this manuscript, based partly on your input, was Major revision. A full copy of the comments to authors is appended, below.

Your assistance and participation in the review process for Scientific Reports is greatly appreciated.

Best regards,

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Referee comments to the authors:

Reviewer #1:
Remarks to the Author:
In this paper, the authors exposed pregnant female rats carrying the lacI mutation-reporter transgene to endocrine disruptor vinclozolin and assessed the frequency of mutations in kidney tissue and sperm recovered from F1 and F3 generation progeny. They found that exposure to vinclozolin in utero does not induced an increase in the frequency of point mutations in F1 generation offspring. However, exposure to vinclozolin in utero predisposes an accelerated accumulation of point mutations detectable in a subset of F3 generation offspring. Based on this discovery, the authors proposed that the existence of "tertiary epimutations" which are initial primary epimutations that promote genome instability leading to an accelerated accumulation of genetic mutations.
It is an interesting discovery that utero exposure to vinclozolin may contribute to increased genetic instability in the F3 generation descendants. However, this is a very preliminary observation with limited evidence to support the proposed hypothesis. We have the following concerns.
1.There is no direct evidence showing that the epigenetic modification caused by vinclozolin in F1 generation related with the transgenerational genetic mutation in F3. They should measure if vinclozolin treatment could cause epigenetic modification (e.g., DNA methylation, histone modification, etc) in F1 generation as compared with controls.
2.They only observed small number of animals in F1 and F3 generations. Because there is significant variation even within each group, there is not enough statistical power to draw a conclusion. They should increase the number of animals in both F1 and F3 generation.

3.For the mutations in kidneys and sperm, it's not clear they measured the kidneys and sperms from same animals or different animals. In another word, do the animals which showed higher mutation frequency in sperm also have higher mutation frequency in kidney? How about other tissues? Is there difference on mutation frequency between genders?
4.What happened to the F2 generation? It will be worth to look at the mutation frequency in F2 generation, to see if there is a transition of mutation frequency from first to third generation.
5.In table 1, we noticed that there is big difference in the total number of plaque-forming units from each animal in VL group. The one who has higher mutation frequency (such as F3VLK6) usually has very low number of total plaque-forming units. Will this influence the accuracy of the frequency counting?
6.In figure 1, what's the overall p-value between CL and VL groups? How to explain the big difference between individual with the VL group? Are these animals the litters from the same set of F1 parents?
7.I am wondering why they didn't use the next generation sequencing (NGS) to sequence the tissues to identify the genetic mutations caused by utero exposure to vinclozolin? The NGS should be more efficiency in detection of genetic mutations.

Reviewer #2:
Remarks to the Author:
Comments to the Authors,

Dr. McCarrey in his manuscript "Tertiary Epimutations-A Novel Aspect of Epigenetic Transgenerational Inheritance Promoting Genome Instability" proposed a very interesting concept of tertiary epimutations, which is the extention of primary and secondary epimutation. The authors observed the mutation frequency were higher in F3 generation progeny rather than F1 and F2. The idea and the strategy were excellent and it would give a great help to understand the relationship between environment exposure and human heredity. I only have several tiny considerations on the study.

1, In the Figure 1, Is there any explanation for 'Panel A have smaller mean, larger error bars (SE) compared with Panel B'? Did you check the visualization of Panel A and Panel B with SD as the error bar? What I am worrying is that only few samples in F3 have large mutation frequency (MF) while majority of them have similar MF, should we check the conclusion more cautiously? Could another exposure treatment or validation study could be assigned?

2, Can you make some interpretation on the ratio of TS/TV in F1 control-lineage is about 2-3 while this ratio become 1-2 in F3 control-lineage? It should not be caused by batch effect? Would there be some reason from biology or technique?

3, In the title, the authors want to claim "Genome Instability" would be the consequence of tertiary epimutations. However, I don't know whether it is suitable to take it as the "Genome Instability" or just consider "Genomic Mutation"? Since the I/D and DBS were not quite significantly different between V and C-lineage, right?

4, The authors mentioned 'Vinclozolin is not directly mutagenic'. Would you mind explaining which drugs could be considered to be directly mutagenic? Is there any necessary to set a such positive control in present study?

5, I just want to confirm is there any previous study have been discovered such F3 higher mutation before? Do you think there would be higher mutation frequency in F4 or more? Another question is why sperm and kidney were selected in the present study?

6, Significant different among the numbers of column Total number of plaque-forming units in Table 1 and Table 2, any explicitly and non-affect reason to caused such difference?

7, Vinclozolin is associated with cancer onset, and the high mutation frequency (Genome-instability) might be the

consequence for pre-cancer event. For other disease, such as diabetes, rheumatology disease, how to demonstrate the tertiary permutations in such scenarios?

Reviewer #3:
Technical Comments to the Author:
In this manuscript John R. McCarrey et al, studied utero exposure of endocrine disruptor vinclozolin in pregnant mice, and reported an interesting, however, currently inconclusive, phenomena - mutation rate increase in a report gene lacI in the F3 generation but not in the F1 by the treatment. The authors thus proposed a model of "tertiary epimutation" - epigenetic alterations not driven by genetic mutation, but would promote genome instability and accelerated accumulation of genetic mutation. The authors have discussed possibility of the involvement of epigenetics modifications as well as chromatin structure in inducing genetic mutations, which if true could be very interesting. However, the major concern is that the authors can't exclude the possibility that genetic mutations may already arise in F0 mice in certain genes, whose function may be involved in genomic stability, which then resulted in accumulated mutation in lacI in F3 generation. Since the study only focused on lacI
gene, thus there is no way to conclude whether the increase of mutation rate in lacI in F3 is due to non-genetic mutations in F0.

It's hard to come up with any sound experimental design to solve the concern. One experiment that the authors can do is to perform deep sequencing in mice from F1 and F3 generations. If the hypothesis is correct, then it should also be true for genes other than lacI. The authors will need to first make sure that there are no mutations caused by vinclolin in genes regulating genomic stability, such as DNA damage repair genes, and then an accelerated mutational rate in whole genome (not only lacI) of mice treated with vinclozolin. Only by increasing the data size by measuring the whole genome, the study can therefore be conclusive. The authors then can further analyze the epigenetic features associated with genes with higher mutation probability versus those resistant to mutation.

In short, the evidence is too preliminary to support the conclusion.